SCIENTIFIC VALITATION OF POTENT ANTI-CERVICAL CANCER, ANTI-TUMOR AND ANTI- OXIDANT ACTIVITIES OF SIDDHA HERBO MINERAL FORMULATION "RASA PARPAM" IN IN-VITRO STUDIES

The dissertation submitted by

Dr.G.DEEPA

Reg. No: 321412101

Under the Guidance of

Dr. V.VELPANDIAN, M.D(S), Ph.D.,

Dissertation submitted to

THE TAMILNADU DR. M.G.R MEDICAL UNIVERSITY

CHENNAI-600032

In partial fulfilment of the requirements

for the award of the degree of

DOCTOR OF MEDICINE (SIDDHA)

BRANCH-II GUNAPADAM

POST GRADUATE DEPARTMENT OF GUNAPADAM

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DECLARATION BY THE CANDIDATE

I here declare that this dissertation entitled **" Scientific Validation of Potent Anti-cervical cancer, Anti- tumor and Anti-oxidant activities of Siddha Herbo Mineral Formulation *Rasaparpam* in in-vitro studies"** is a bonafide and genuine research work carried out by me under the guidance of **Dr.V.Velpandian, M.D(S), Ph.D.,** Post Graduate Department of *Gunapadam*, Govt.Siddha Medical College, Arumbakkam, Chennai-106 and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

Date: Signature of the Candidate

Place: Chennai G.DEEPA

GOVT. SIDDHA MEDICAL COLLEGE,
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CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **" Scientific Validation of Potent Anti-cervical cancer, Anti- tumor and Anti-oxidant activities of Siddha Herbo Mineral Formulation *Rasaparpam* in in-vitro studies"** is submitted to The Tamilnadu Dr.M.G.R. Medical University in partial fulfillment of the requirements for the award of degree of M.D (Siddha) is the bonafide and genuine research work done by **Dr. G.DEEPA** under my supervision and guidance and the dissertation has not formed the basis for the award of any Degree, Diploma, Associateship, Fellowship or other similar title.

Date: **Signature of the Guide**

Place: Chennai

GOVT. SIDDHA MEDICAL COLLEGE,
ARUMBAKKAM, CHENNAI-106

ENDORSEMENT BY THE HOD AND PRINCIPAL OF THE INSTITUTION

This is to certify that the dissertation entitled **" Scientific Validation of Potent Anti-cervical cancer, Anti- tumor and Anti-oxidant activities of Siddha Herbo Mineral Formulation *Rasaparpam* in in-vitro studies"** is a bonafide work carried out by **Dr.G.DEEPA** under the guidance of **Dr.V.Velpandian, M.D(S**), **Ph.D.,** Post Graduate Department of *Gunapadam*, Govt.Siddha Medical College, Chennai - 106.

Signature of the HOD **Signature of the Principal**

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ABBREVIATION

ALT - Alanine amino transaminase

ALP - Alkylanine Phosphatase

AST - Aspartate Amino Transferase

ANOVA - Analysis of Variation

BUN - Blood Urea Nitrogen

CT - Computed Tomography

COX - Cyclo oxygenase

CMC - Carboxyl Methyl Cellulose

CAMP - Cyclic Adenosine Monophosphate

CPCSEA - Committee for the Purpose of Control and Supervision of Experimental Animals.

DMEM - Dulbecco's Modified Eagle's Medium

DPPH - 2,2-diphenyl-1-picrylhydrazyl

DMSO - Dimethyl sulfoxide

DNA - Deoxyribo Nucleic Acid

DC - Differential Count

EBRT - External Beam Radiation Terapy

EDTA - Ethylenediamine Tetra acetic Acid

EDX - Energy Dispersive X-ray Spectrometry

ENU - N-ethyl-N-nitrosurea

ERK assay - Extracellular signal-regulated kinases

EUA - Examine Under Anesthesia

FDG-PET - F-18 Fluoro-2-deoxy-D-glucose

FAD- Assay - Flavine Adenine Dinucleotide

FTIR - Fourier Transform Infrared Spectrometry

GOT - Glutamate Oxaloacetate Transaminase

GPT - Glutamate Pyruvate Transaminase

HDR - High –dose rate

HPV - Human Papilloma Virus

HSV2 - Herpes Simplex Virus type -2

HDL - High Density Lipoprotein

ICP-OES - Inductively Coupled Plasma Optical Emission Spectrometry

IAEC - Instituional Animal Ethical Committee

ICMR - Indian Council of Medical Research

LDL - Low Density Lipoprotein

LDR - Loe dose rate

LLETZ - Large Loop Excision of the Transformation Zone

LD50 - Lethal Dose

MCV - Mean Corpuscular Volume

MRI - Magnetic Resonance Imaging

MTT - 3-(4,5-Dimethyl thiazol-2-yl)-2,5-Diphenyl Terazolium Bromide

MCH - Mean Corpuscular Haemoglobin

MCHC - Mean Corposcular Haemoglobin Concentration

NCRP - National Cancer Registry programme

OECD	-	Organisation for Economic Corporation and Development
PCR	-	Polymerase Chain reaction
PCV	-	Packed Cell volume
RBC	-	Red blood cells
SEM	-	Scanning Electron Microscope
SEM	-	Standard Error Mean
SGOT	-	Serum Glutamate Oxaloacetate
SGPT	-	Serum Gluatamate pyruvic transaminase
VEGF	-	Vascular endothelial growth factor
VLDL	-	Very Low density Lipoprotein
WDS	-	Wavelenth Dispersive Spectroscopy
WBC	-	White blood cells
WHO	-	World Health Organization

1. INTRODUCTION

Cancer is characterized by never-ending and uncontrolled anarchic cell proliferation. This anarchic proliferation of abnormal *cells is* opposed to the controlled, harmonious proliferation of normal tissues which only occurs to repair damaged or worn tissues. Carcinogenic factors (tobacco, alcohol, pollutants, chemicals, radioactivity, vaccines, viruses, aging, stress, violence, despair, etc.) produce mutations or methylations.

Cancer is the second leading cause of clinical mortality in developed countries. Cancer and tumors are the result of altered, excessive and invasive cell reproduction in other nearby healthy tissues, their development can be originated from diverse causes, like genetics, to the consumption of potentially toxic and harmful foods. In India, tumors (neoplasms) are the third cause of mortality. In this category, malignant tumors of the cervix are the first cause of female death. Cervical cancer is the second most common cancer in the women worldwide and the leading cause of cancer deaths among women in developing countries[(1)].

Sexually transmitted human papilloma virus (HPV) infection is the most important risk factor for cervical intraepithelial neoplasm and invasive cervical cancer[(2)].HPV serotypes 16 and 18 account for nearly 76.7% of cervical cancer in India. Warts have been reported in 2–25% of sexually transmitted disease clinic attendees in India; however, there is no data on the burden of anogenital warts in the general community[(3)].

Every year in India, 122,844 women are diagnosed with cervical cancer and 67,477 die from the disease[(4)].

HPV is a virus that can lead to conditions as harmless as a wart and as deadly as cervical cancer. Although there is no cure for HPV, there are preventative measures and herbs with antiviral properties that might be able to reduce the adverse effects and spread of the disease. Human papilloma virus (HPV) is a virus that can infect human mucous membranes and epidermis, which can lead to cancers of the vagina, anus, cervix, vulva and penis. There are more than 100 different types that are usually divided into those that cause warts, those that cause cancer, and those that have no symptoms at all (which are essentially harmless). Some types of viruses can be

sexually treated (like the strain that causes genital warts) and some have a chance to get rid of on their own within two years of 90 percent. The Pap smear test is intended to detect the cells that can develop in the cervix of the uterus as a result of HPV.

In Modern system of medicine, the main strategy of treating patients with cancer are surgery, radiation and chemotherapy[(5)]. Most patients unfortunately seek Siddha system of medicine or other traditional systems of medicine after a failure of chemotherapy and very aggravated situation most of the time.In Siddha system of medicine, plants, metals and mineral based medicines have been used since ancient times to treat and prevent different types of cancer.

Cancer and tumors, whether benign or malignant, are a condition that can be treated with Siddha medicines, either in combination with other treatments or even medicines, as the Siddha medicine does not interfere with any other anti-cancer alternative and can certainly support the patient in a positive way in the healing process.

In this regard, effective therapy against cancer, thousands of research on plants and other metallo- mineral preparations from Siddha system of medicine to find potent anti-cancer agents.These medicine have treated many diseases across all over India for thousands of years including cancer. They slow down or block cancer proliferation and stimulate the body's anti-cancer defenses.

In Siddha treatment, there are number of medicines to cure cervical cancer in a non-complicated way. There are also number of literature works to facilitate this. In particular, a potent medicine "*RASAPARPAM*" is mentioned in the classical Siddha book " Aathma Rakshamirtham Ennum Vaithiya Saara Sangiragam" written by Kandhasamy Mudhaliyar.

Mercury:

In Siddha medicine, mercury is extensively used in the preparation of medicines after purification. Mercury is also used to in the alchemical and rejuvenation medicine preparations.

RASA means elixir of life and the word is attributed to the most important factor responsible for life in various fields of life of knowledge. In the herbal domain

it is the essence of a substance. The same meaning is implied to mercury by denoting it as rasa (ரஸ) of the mineral kingdom, or in other words the metal of utmost important having distinct properties and unique nature is given the term "Rasa"[6]

Even though pure herbal based medicines are effective, mercury based medicines like *Parpam, Mezhugu and Chendhooram* are prepared for the following reasons [7]

- Highly potent
- Fast action
- Vast utility
- Easy to consume
- Smaller dose is enough
- Acts as rejuvenation medicine

Mercury is the most superior among the other metals. Mercury is compared to lord Siva because it has [8] [9]

- Creative power
- Protective power
- Destructive power

PARPAM

In Siddha, *Parpam* is considered as a higher order of medicine. Even a small dose of *Parpam* can used to cure chronic diseases, complicated diseases and life threatening diseases like cancer etc.,

The most super natural power present in *Parpam* is its longevity, it has a longevity of 100 years.

The peculiarities of *Parpam* and its significance are expressed by Theran in his words are as follows[10]

‘‘ வீரத்து மிக்கவை பற்பங்களே- பரி
காரத்து மிக்கவை பற்பங்களே
பாருக்குள் மானிடர் நோய்போக- வரு
பண்டிதருக்கெல்லா மாமோகம்
வீரகடாரி - பிணிக்கொரு
பாரகுடாரி - விசைபெறு
தீரதடாரி - வினையுடு
சூரிக்குழு நேரொத்தது
மேருக்கினை பாரப்புறம். “

- தேரன் தரு.

This Herbo mineral classical Siddha drug *Rasaparpam* is the best drug against cervical cancer. The present study was carried out with the objective of validating the safety and efficacy of *Rasaparpam* and its potent cytotoxic activity in culture of HeLa and SiHa cells.

2. AIM AND OBJECTIVE

AIM:

To justify the ancient Siddha drug for management of cervical cancer with its ultimate formulation and give good progress to the people affected by cevical cancer. The purpose of the present study was aimed to validate the activity of ***Rasa Parpam*** for its efficacy in managing Cervical cancer through pre-clinical aspects.

In the present medical world, there is a need for proper treatment for Cervical Cancer. The aim of this study is validation of a new drug for the management of Cervical cancer.

OBJECTIVES

The key objectives of the study are,

- Having a collective review of the literature.
- Preparing the drug according to Siddha classical text.
- Subjecting the drug to physico-chemical standardization.
- Analyzing the drug chemically for detection of acid and basic radicals.
- Focusing the drug for analytical assessment.
- Studying the toxicity profile (acute and 28-days repeated oral toxicity) of *Rasa Parpam* according to OECD guidelines.
- Determining the pharmacological activity (*In-vitro* Anti-cancer activity) of *Rasa Parpam.*like,
    - Anti-cancer activity in HeLa cell line model by MTT assay,
    - Anti –tumor activity in HeLa and SiHa cell line models,
    - Anti- oxidant activity by DPPH assay.
- Analyzing all the above study results to validate the benefits of *Rasa Parpam* .

3. REVIEW OF LITERATURE

3.1. Drug Review

3.1.1. Siddha Aspect

1. *Rasam*

Synonyms

Mercury or Quick Silver

Chemical name

Hydragyrum

Mercury (Rasam) comes under the classification of *Pancha Sootham*. The term ' *Pancha Soothaas*' refer the five types of Mercury (*Rasam*). It has many connotations such has *Sootham, Punniyam, Karpam, Satthu, Bharatham, Easan, Pootham* according to "*Dasanga Nigandu*".

Mercury is obtained from its ores in countries like Spain, California, Russia, China and Japan. It is separated from its ore Cinnabar.

Agonist to Mercury:

Abragam – *Mica*

Kaareeyam – *Lead*

Kandhagam – *Sulfur*

Velli – *Silver*

Sembu – *Copper*

Thuttham – *Zinc*

Thalagam – *Arsenic pentasulphide*

Veeram – *Perchloride of Mercury*

Antagonist :

Singi – *Plumbioxidum*

Vellaipadanam - *White Arsenic*

Irumbu – *Iron*

Kaantham – *Magnet*

Soodan – *Camphor*

Pooram – *Sub chloride of Mercury*

Nimilai – *Bismuth*

Pooneeru – *Fuller's earth*

Classification of Mercury

It is classified into 5 types. It is made up of five basic elements and is classified into 5 types according to the basic elemental ratio.

1. *Rasam* – It is red in colour and without any ill effects
2. *Rasendhiran* –This is black in colour
3. *Sootham* – This is yellow in colour contains impurities. Hence it should be purified and detoxified before use.
4. *Misaragam* – It is multicoloured. This also needs to be purified and detoxified before use.
5. *Bharatham* – It is white in colour and found in commercial grades commonly.

Mercury has the following properties

Action:

Vitalizer, Tonic, Laxative, Diuretic, Neutralises bile,Silagogue, Anti-inflammatory, cures venereal diseases (Meganasini).

Taste:

Six tastes – dominated by sweet

Potency:

Hot and cold (both specialities)

Further it facilitates to attain the following eight folded " Siddhis" (*Attama Siddhi*)

Anima, Mahima, Karima, Lahima, Prapthi, Prakamiyam, Esatthuvam, Vasithuvam.

***Dhosas* (Impurities) of Mercury:**

It has 2 types of impurities,

1. *Dhosa*
2. *Sattai*

Dhosa:

There are 8 types of impurities in Mercury which cause various diseases are as follows,

1. *Undeenam – Throbbing pain (Soolai)*
2. *Kowdilyam – disease of head (Kapaalanoi)*
3. *Anavartham – Maniac illness (Brammai)*
4. *Sangaram – Spermatorrhoea (Thathunattam)*
5. *Sandathavam – Distress*
6. *Panguthuvam – Morbid thirst and leprosy*
7. *Samalathvam – Fever and syncope*
8. *Savishathvam – Emaciation – loss of weight*

Sattai:

7types

1. *Naagam – Piles*
2. *Vangam – Skin disease*
3. *Malam – Ignorance*
4. *Vidam – Death*
5. *Agni – Morbid thirst polydypsia*
6. *Giri – Distress*
7. *Sagalam – Spermatorrhoea*

8 types of *Dhosas* and diseases according to Therayar,

1. *Sarppam – Blister*
2. *Vangam – Skin diseases*
3. *Ganthi – Heat*
4. *Vanni – Burning sensation*
5. *Sanjalam – Discolouration*
6. *Malam – Spermatorrhoea*
7. *Kaalam – Death*
8. *Mantham – Syncope*

7 types of *Dhosas* and diseases according to Agathiyar,

1. *Nagam – Bad odour*
2. *Vangam – Skin diseases*
3. *Ganthi – Thirst*
4. *Vanni – Spermatorrhoea*
5. *Sanjalam – Death*
6. *Vidamum – Toxic disease*
7. *Logam – Aphrodisiac*

General characters of Mercury:

"விழிநோய் கிரந்திகுன்மம் மெய்சூலை புண்குட்
டழிகாலில் விந்துவினால் அத்தை - வழியாய்
புரியு விதி யாது புரியினோ யெல்லாம்
இரியுவிதி யாது மில்லை "

- குணபாடம் தாது-சீவ வகுப்பு

Proper use of Mercury as a medicine cures the following diseases. They are disease of eyes, syphilis, eight types of peptic ulcers (*gunmam*), throbbing pain (*soolai*), chronic ulcers (*perumpun*), leprosy (Hansen's disease).

2 types of properties:

1. Beneficial properties
2. Harmful effects

Beneficial properties:

- It purifies blood
- It improves quality of blood and semen
- Stimulates appetite
- Kills the micro-organisms and cures the wound.
- It cures the diseases of internal and external organs of the body
- It improves memory power, cures Amnesia
- It strengthens the nerve plexuses
- It develops wisdom through concentration of mind
- It prevents senility and increases the life span.

Harmful effects:

Unpurified Mercury causes,

- Bleeding
- Dropsy
- Anaemia
- Excessive body heat
- Sweating
- Diarrhea
- Thirst
- Flatulence
- Blabbering
- Skin diseases
- Burning sensation of the limbs
- Diseases of head
- Fever
- Shivering
- Hiccough etc.,

Purification and detoxification of Mercury:

Mercury - 35grams

Brick powder - Required quantity

Turmeric powder - Required quantity

Acalyphaindica juice - 1.3 lit

Mercury was triturated with finely powdered brick and then turmeric powder for one hour respectively and washed with water. Then Mercury is boiled with the juice of *Acalypha indica* till the juice completely evaporates. We get purified Mercury.

Some preparations of Mercury:

- *Soothakaruppu*
- *Rasa mezhugu*
- *Rasa thailam*
- *Megaviranakalimbu*
- *Rasa kuligai*

Antidotes for Mercurial poisoning:

- If the Mercurial poison affects the gluteal region, root bark of dye plant (*Caesalpiniasappan-Sayapattai*) is powdered and given along with jaggery.
- When teeth are affected the stem juice of ivy gourd (*Coccinaindica- kovai*) may be squeezed on the tongue.
- If there is burning sensation in limbs, urticaria, dryness of the throat and the patient is unconscious, Barmuda grass root stalk *(Cynodondactylon-Aruganizhangu*) is triturated and dissolved in goat's milk or cow's milk or butter milk or cotton seed milk is filtered and administered[(11)]

3.1.2. Modern Aspect

Mercury

Mercury is not less than 99.5 percent of Hg. It occurs naturally as a Sulphide ore called Cinnabar HgS. It also occurs in small globules disseminated through rocks and as amalgam of Silver and Gold.

Chemical properties of Mercury [12]

Atomic number	-	80
Atomic mass	-	$200.59 g.mol^{-1}$
Electro negativity	-	1.9
Density	-	$13.6 g.cm^{-3}$ at $20^{\circ}c$
Melting point	-	-38.9ºc
Boiling point	-	$356.6^{\circ}c$
Radius	-	0.157nm
Ionic radius	-	0.11nm (+2)
Isotopes	-	12
Electronic shell	-	$[Xe]4f^{14}$
Standard potential	-	+0.854V

Preparation:

- It is obtained by roasting Cinnabar in a current of air $HgS+O_2 \rightarrow Hg+SO_2$
- The free Mercury gets liberated. It may be either purified by volatilization or chemically by dropping Mercury into a column of dilute Nitric acid for removing basic impurities.

Properties:

It is shiny silvery white in nature. Heavy liquid easily divisible into globules and extremely mobile, easily volatilizes on heating. It boils at 359.58°C.

Almost insoluble in water, alcohol and HCl. It dissolves in cold and dilute Nitric acid, giving Mercurial nitrate and Nitric oxide[13].

$$6Hg+8HNO_3 \rightarrow 3Hg_2(NO_3)2+2NO+4H_2O$$

Mercury salts

The most important Mercury salts are Mercuric chloride $HgCl_2$ (corrosive sublimate - a violent poison), Mercurous chloride Hg_2Cl_2 (Calomel, still used in medicine occasionally), Mercury fulminate $(Hg(ONC)_2$, a detonator used in explosives) and Mercuric sulphide (HgS, Vermillion, a high-grade paint pigment)

Applications [14]

- Mercury as a metal has many uses. Because of its high density it is used in barometers and manometers. It is extensively used in thermometers, due to its high rate of thermal expansion that is fairly constant over a wide temperature range.
- Its ease in amalgamating with gold is used in the recovery of gold from its ores.
- Industry uses Mercury metal as a liquid electrode in the manufacture of chlorine and Sodium hydroxide by electrolysis of brine.
- Mercury is still used in some electrical gear, such as switches and rectifiers, which need to be reliable, and for industrial catalysis.
- Much less Mercuryis now used in consumer batteries and fluorescent lighting, but it has not been entirely eliminated.
- Mercury compounds have many uses. Calomel (Mercurous chloride, Hg_2Cl_2) is used as a standard in electrochemical measurements and in medicine as a purgative.
- Mercuric chloride (corrosive sublimate, $HgCl_2$) is used as an insecticide, in rat poison, and as a disinfectant.
- Mercuric oxide is used in skin ointments.
- Mercuric sulphate is used as a catalyst in organic chemistry.
- Vermilion, a red pigment, is mercuric sulphide; another crystalline form of the Sulphide (also used as a pigment) is black.
- Mercury fulminate $Hg(CNO)_2$ is used as a detonator.

Mercurial preparations:

- Mercury with Chalk (Grew powder)
- Yellow Mercuric Oxide (HgO)
- Mercuric Oxide
- Oleated Mercury
- Mercurous Chloride (HgCl-Calomel)

Tests for Purity:

It has been tested for weight per ml (at 25°C is about 13.5g).Non-volatile matter residue at 300°C (not more than 0.02%w/w).

Assay:

An accurately weighed quantity (0.49g) is dissolved in equal parts (20ml) of water and Nitric acid. It is heated gently until the solution becomes colourless. The solution is then diluted with water (150ml) and sufficient quantity of Potassium permanganate is added till a permanent pink colour is obtained. A trace of ferrous sulphate to discharge pink colour is added. Then the solution is titrated with standard 0.1N Ammonium thiocyanate (1ml of 0.1N Ammonium thiocyanate =0.01003g), using Ferric Ammonium sulphate as indicator. The temperature during the titration should not exceed above 20°C.

$3Hg+8HNO_3 \rightarrow 3Hg\ (NO_8)2+4H_20+2NO\uparrow$

$Hg\ (NO_3)2+2NH_4SCN \rightarrow 2NH_4NO_3+Hg\ (SCN)2$

Fig.No.1. ELEMENTAL MERCURY

Uses

It is a pharmaceutical aid and for preparing Mercury with chalk .Formerly metallic Mercury was used therapeutically as a cathartic and parasticide. Almost all the salts of Mercury with the exception of the Sulphide is poisonous.

1. Mercury with chalk (Grew powder)

- It is having 31 -35% w/w of Mercury and 62-70% w/w of $CaCO_3$
- It is used as a purgative (Dose 60-300mg)

2. Yellow mercuric Oxide (HgO)

- It has not less than 99.5% HgO. It is used as a mild anti-septic, anti-infective and anti- bacterial agent.

3. Mercuric Oxide:

- It contains not less than 95% but not more than105% w/w of the stated amount of yellow Mercuric oxide
- It is used in ophthalmology, 1% ointment to treat mild inflammatory conditions for the treatment of blepharitis and conjunctivitis.

4. OleatedMercury:

- It has the equivalent of 20% of yellow Mercuric oxide
- It is used as an anti-infective.

5. Mercuric chloride (HgCl - Calomel):

- It is not less than 99.6% of HgCl
- It has been used for centuries as a cathartic but recently it is replaced by other drugs [15].

Calomel is insoluble in gastric juice and is not absorbed from the stomach. It gets absorbed in the intestine by the alkaline pancreatic juice where it slowly gets dissociated into Mercury and irritant Mercuric compounds which exerts a cathartic action[16].

Siddha Aspect

2. *Gandhagam* (Sulfur)

Chemical name

Sulfur

Synonyms

Kaarizhainnaatham, Paraiveeriam, Atheethaprakaasam, Peejam, Sakthi, Sakthipeesam, Selvivindhu, Naatham, Naatram, Deviuram.

General properties

Taste:

Gandhagam is bitter and astringent in taste.

Actions:

- Laxative
- Tonic
- Antiseptic

It increases the various secretions of the body including skin. When used in high doses, it causes diarrhea.

Types

*Gandhagam*is divided into four types depending upon their colour, appearance and properties.

1. White colored Sulfur
2. Red colored Sulfur
3. Golden yellow colored Sulfur
4. Black colored Sulfur.

In addition, gooseberry Sulfur and stick Sulfur (*Vaanagandhagam)* have been mentioned in most of the text books of ancient Siddha medicines. Gooseberry Sulfur is often used in medicinal preparations.

General characters of Gooseberry Sulfur (*Nellikai gandhagam)*

"நெல்லிக்காய்க் கந்திக்கு நீள்பதினெண் குட்டமந்தம்
வல்லை கவிசைகுன்ம வாயுகண்ணோய் - பொல்லா
விடக்கடிவன் மேகநோய் வீறுசுரம் பேதி
திடக்கிரக ணீகபம்போந் தேர்"

- பதார்த்த குண சிந்தாமணி.

It is used in the treatment of 18 types of skin diseases, liver enlargement, abdominal distension, eye diseases, chronic venereal diseases, chronic diarrhea, gastric ulcer, poisonous bites, fever, and chronic dysentery.

Method of purification

Sulfur is placed in an Iron spoon. A small quantity of cow's butter is added and the spoon is heated till the butter melts, this mixture is poured in inclined position in cow's milk. This procedure is repeated for 30 times to get purified Sulfur. Each time fresh milk is to be used[17].

Preparations of Sulfur

- *Kandhagaparpam*
- *Sarvavidathodarikuligai*
- *Kandhagachendhooram*
- *KandhagaMezhugu*
- *KandhagaMaathirai*
- *Kandhaga Thylam*
- *Kandhaga Vadagam*
- *Kandhaga Rasayanam*

3.1.2. Modern Aspect

Sulfur

Sulphur or Sulfur is a Greek word which means "to burn". Sulfur is a chemical element with the symbol S. It is a plentiful, multivalent non-metal. It occurs in nature as the pure element and as Sulfide and Sulfate minerals. Sulfur is referred to in the Bible as brimstone (burn stone) in English.

History:

- Sulfur was discovered by Chinese before 2000BC and is recognized as an element by Antoine Lavoisier in 1777.
- Sulfur is mentioned in *Bible* and was best known for destroying Sodom and Gomorrah. It was also known to the ancient Greeks and burnt as a fumigant. Sulfur was mined near Mount Etna in Sicily used for bleaching cloth and preserving wine, both of which involved burning it to form Sulfur dioxide and allowing this to be absorbed by wet clothes or the grape juice. For centuries, sulfur along with Mercury and salt, was believed to be a component of all metals and formed the basis of alchemy whereby one metal could be transmuted into another.
- Antoine Lavoisier thought that Sulfur was an element, but in 1808 Humphry Davy said it contained hydrogen [(18)].

Properties [(19)]

General properties:

Symbol	-	S
Number	-	16
Element category	-	polyatomic nonmetal

Physical properties:

Phase	-	solid
Density	-	1.96 g·cm−3
Liquid density at M.P	-	1.819 g·cm−3
Heat of fusion	-	1.727 kJ·mol−1
Heat of vaporization	-	45 kJ·mol−1
Molar heat capacity	-	22.75 J·mol−1·K−1
Electronegativity	-	2.58 (Pauling scale)

Chemical properties:

Solubility	-	insoluble in water
Vanderwaals radius	-	0.127 nm
Ionic radius	-	0.184 (-2) nm; 0.029 (+6)
Isotopes	-	5
Electronic shell	-	[Ne] 3s23p4
Standard potential	-	0.51 V

Image explanation

- The alchemical symbol for sulfur is shown against a 'fire and brimstone' background.

Appearance

- There are several allotropes of sulfur. The most common appears as yellow crystals or powder.

Fig.No.2. Sulfur

Uses

- Sulfur is used in the vulcanisation of black rubber, as a fungicide and in black gunpowder. Most sulfur is however used in the production of sulfuric acid, which is perhaps the most important chemical manufactured by western civilisations. The most important use of sulfuric acid is in the manufacture of phosphoric acid to make phosphates for fertilisers.

- Mercaptans are a family of organosulfur compounds. Some are added to natural gas supplies because of their distinctive smell, so that gas leaks can be detected easily. They are also used in Silver polish and in the production of pesticides and to eradicate weeds.
- Sulfites are used to bleach paper and as preservatives in many foodstuffs. Many surfactants and detergents are sulfate derivatives. Calcium sulfate (gypsum) is mined on the scale of 100 million tonnes each year for use in cement and plaster.

Biological role

Sulfur is essential to all living beings. It is taken up as Sulfate from the soil or sea water by plants and algae. It is used to make two of the essential amino acids needed to make proteins. It is also needed in some co-enzymes. The average human body contains 140 grams and takes in about 1 gram a day, mainly in proteins.Sulfur and Sulfate are non-toxic. However, Carbon disulfide, Hydrogen sulfide and Sulfur dioxide are all toxic. Hydrogen sulfide is particularly dangerous and can cause death by respiratory paralysis.

Natural abundance

Sulfur occurs naturally as the element, often in volcanic areas. This has traditionally been a major source for human use. It is also widely found in many minerals including Iron pyrites, Galena, Gypsum and Epsom salts.

Elemental Sulfur was once commercially recovered from wells by the Frasch process. This involved forcing super-heated steam into the underground deposits to melt the Sulfur, so it could be pumped to the surface as a liquid.

Modern Sulfur production is almost entirely from the various purification processes used to remove Sulfur from natural gas, oil and tar sands. All living things contain Sulfur and when fossilised (as in fossil fuels) the Sulfur remains are present. If unpurified fossil fuels are burnt, Sulfur dioxide can enter the atmosphere, leading to acid rain [(20)].

Siddha Aspect

3. *Urginea indica - Narivengayam*

Synonyms

Kaateerulli, Kaatuvengayam.

Vernacular Name

English : Bulb of Indian Squill

Telugu : Nakka-vulli-gadda

Malayam : Kaatulli

Kannada : Adavi-neerulli

Hindi : Jangli- pilyx

Part used

- Bulb

Characters

Suvai – Kaippu

Thanmai – Veppam

Pirivu _ Karppu

Actions

- Expectorant
- Stimulant
- Emmenagogue
- Diuretic

General characters

"நஞ்சோடு சீதகபம் நாடாதி ரைப்புமறும்
பஞ்சாக மூல பகந்தரம்போம் - பஞ்சணைசேர்
வீட்டுள்ளிருக்கும் வினோத மடமயிலே!
காட்டுள்ளி யிங்கிழங்கைக் கண்டு"

- அகத்தியர் குணவாகடம்

Uses

- Snake poison, Cough, Asthma, Piles and Fistula[21].

Fig.No.3. *Urginea indica*

Botnanical Aspect – *Urginea indica*

Taxonomical classification:

Plant ID **:** 0132

Family : Liliaceae

English Name : Indian Squill, True Squill, Sea Onion.

Common Name **:** Junglipyaz

Classification [22]:

Kingdom : Plantae

Sub kingdom : Tracheobionta

Division : Mangnoliophyta

Class : Magnoliopsida

Subclass : Liliidae

Order : Liliales

Family : Liliaceae

Genus : Urginea

Species : Indica

Part used:

- Plant and Bulb

Description of the Plant:

- A bulbous herb; with truncate bulbs, 5-10 cm long, ovoid.
- Leaves radical, appearing after the flowers, 15-45 cm long, linear, acute.
- Scape erect,brittle 30-45 cm long and 4 to 6 mm in diameter at the base.
- Flowers small, dingy brown, very distant, on slender, laxly flowered racemes, 15-30 cm long.
- Fruits are capsules 1.3-2 cm long, ellipsoid, tapering to both ends, the cells 6 to 9 seeded.
- Seeds are flattened and black.

Medicinal properties

Bulb

- Anthelmintic
- Alexiteric
- Expectorant
- Cardiac
- Deobstruent
- Emmenagogue

Plant:

- Cyanogenetic
- Expectorant

Uses

Bulb is diuretic and cardiac stimulant; used chiefly as a powerful expectorant in the treatment of cough, especially in chronic bronchitis and asthma; in large doses it is emetic and cathartic and may cause cardiac depression. Its cardio–tonic activity

resembles that of digitalis. The extract of bulb also shows hypoglycaemic and anticancer activity.

Juice of the herb is taken with honey. It controls irregular Mensturation. The bulb is used for dropsy cure. Becouse of diuretic properties, it helps to increase the urine volume. Bulb also cures headaches and diseases of the nose. This is also used to treat various other diseases such as Rheumatism,chronic nephritis, ringworm, leprosy and scabies.

ChemicalConstituents

Bulb

- Cardiac glycosides, Hentriacontanol, Octacosanoic acid. It also contains various Flavonoids including quercetin derivatives and kaempferol polyglycosides, Sinistrin, Mucilage and Calcium oxalate.
- Fresh squill yields at least two Glycosides, Scillaren-A and Scillaren-B[(23)].

Root, bulb and leaf

- Sitosterol, Stigmasterol and Campasterol.

Tuber

- Scillarenin

Habitat

It is found growing in sandy places, especially, the sea coast in most of the Mediterranean districts, being abundant in southern Spain, found in Portugal, Morocco, Algeria, Corsica, Southern France, Italy, Malta, Dalmatia, Greece, Syria, Canary island and the cape of good hope, India, Africa. It is often grown under fig trees in the Italian Riviera,& grown in many botanical gardens and it is stated recorded as cultivated plant in England in 1648 & in oxford botanical gardens.

Ditribution:

Chittagong, Cox's Bazar[(23)].

3.2. Disease Review

3.2.1. Siddha Aspect of the Disease

Siddha system of medicine deals cancer and its treatment widely. In ancient Siddha literature cancer is explained as in the name of ***Putru*** which gives the direct meaning and as *Arpudham* and *Vanmeegam*. For the pupose of diagnose and treatment following reference books evaluates great ideas about Cancer.

1. *YugiVaidhiyaChinthamani*
2. *AnubogaVaidhiyaNavaneetham*
3. *Pulipani 500*[24]
4. *Agathiya Vaidhiya Vallathi*
5. *Agathiyavaithiyavallathi 600*[25]
6. *Anubogavaidhiyabhramaragasiyam*
7. *Agathiyavaithyagandam*

The great *Siddhar* Agatthiya in his *Vaidhiya Vallathi 600* had explained cancer and its different categories.

"போக்குமே திமிர்வாத மண்டைசூலை புற்றுடனே

கேட்குமே அரையாப்பு பவுத்திரத்தைக் கேள்

தண்டு சூலையொடு லிங்கப் புற்றே"

- அகத்தியர் வைத்திய வல்லாதி

"நாமப்பா கருங்கிரந்தி யோனிப்புற்று

ஆமப்பா கருவழிக்குங் கிரந்தி லிங்கப்புற்று"

- யூகி வைத்திய சிந்தாமணி

"இருபுடரி நுனி நாசி சிலந்தி புற்று

தடர்சிலந்தி படர்சிலந்தி அல்குல் புற்று

பின்கரப்பான் முங்கரப்பான் அண்டப் புற்று

துணிவாத உந்திப்புண் துடையில் புற்று

கீழ்நாக்கு மேல்நாக்குப் புற்றுப் போமே"

- புலிப்பாணி 500

The unique saint Pulipani also dealt with different type of cancer in his *Pulipani 500.*

"ஓமேனி குழிப்புற்று யோனிப்புற்று

ஒளிவான இடிப்புற்று கன்னப்புற்று"

- யூகி வைத்திய சிந்தாமணி

In this medical system of life, the cancerous growth and tumors are headed as *Arputha viranangal* and *Arputha kattigal.*

According to *Yugimamunivar Vaithiya Chinthamani* 800 part I, some kinds of cancer clarified under different systemic diseases.

Yugi classifications of diseases are compared with Modern system of medicine by names of symptoms for quick and easy approach[(26)].

For example,

- *Ukkarasoolai* is understand as prostatic cancer
- *Vilperuvayiru* can be compared with Testicular cancer
- *Mamisamagotharam* and *Kalperuvayiru* as cancerous growth within the abdomen.

Cancer is considered as *Vippuruthi.*

Types of *Vippuruthi*

Vippurithi is classified into 7 types,

1. *Karppa Vippuruthi*
2. *Kuvalai Vippuruthi*
3. *Vatha Vippuruthi*
4. *Pitha Vippuruthi*
5. *Sutthuma Vippuruthi*
6. *Santhu Vippuruthi*
7. *Odu Vippuruthi*

Appearance

Cancerous growth appear like,

- Solid tumor - *Kazhalaikatti*
- Spreading ulcer
- Initially like warts then growth develops as turtle shell with oozing .
- Hyper pigmentation of skin, affects hair follicles and destroys entire body.

Classification:

Cancer classified into 3 types according its spreading nature (Metastasis)

- Skin and its structures
- Muscles
- Blood vessels and bones

Causes

- Vitamin and mineral deficiency
- Increased sexual activity
- Prolonged starvation
- Excessive use of tobacco
- Excessive intake of hot and spice food
- Taking excessive amount of salt and pungent food stuffs
- Taking large quantity of fish and meat
- Sleeping in the day time.

Symptoms

Symptoms vary depending on the particular type of cancer.

Yoni Putru

Yoni means birth passage. This is cervix of uterus. So the cancer of cervix is known as Yoniputru. It is also called *Karuppai kazhunthu putru.*

Symptoms

- Growth in cervix appearing like small grains.
- Discharge like honey
- Hardening of surface
- Profuse bleeding
- Constipation
- In some patients discharges with intolerable foul smell.
- Oliguria and anuria
- Administration of diuretics causes haematuria.

Discharges classified into 3 types

1. Viscous yellowish discharge
2. Yellow discharge with mucous
3. Bloody discharge due to non healing cervical ulcer and cancer of cervix.

The SiddharYugi in his Vaidhiya Sinthamani mentioned the symptoms of *Yoniputru* in different types as follows,

Kuruthiyoni (27a)

"திறமான வுபத்திரவ மதிகங் காணும்
தெளியாத ரத்தமுடன் சீழ்நீர்ப் பாய்ச்சல்
கறமான நுரையுடனே நோயுண்டாகும்
கடினமாஞ் சதையுடனே குத்தல் காணும்
நிறமான மஞ்சளுடன் கசரோ கந்தான்
நிலையாது வல்குலிலே புழுவோ மெத்த
மலமான சொல்லதுவு முளுத்தாற் போல
மஞ்ஞையா னிறம்போல் மசக்கும் பாரே."

- யூகி முனி

Profuse bleeding with mucous, micro ulcers like pits on the wall of cervix, discoloration of os.

Kuruthicheezhyoni[28]**:**

"பாரேதாமன் வேதனை மிகவுண்டாகும்
பாங்கான சீழுடன் ரத்தங் காணும்
சீரேதான் ஒழுக்குடன் நாற்றமாகும்
சிதறியே பலபேத வண்ணங் காட்டும்
நேரேதா நிதம்பத்தின் ஸ்தனந் தன்னில்
நெடிதான ரோகத்தை மேவச் செய்யும்
வேரேதான் சொன்னபடி சிகிச்சா சாரம்
விரித்திட்டர் யூகிமுனி விளக்கந் தானே."

- யூகி முனி

Bleeding with mucous sometimes in multicolor, foul smelling discharge with bad odour, spread to whole uterus.

Mamisamagotharam[27b]**:**

"போக்கான மாமிசந்தான் வளர்ந்து மீறி
பொருமியே அடிவயிற்றில் கல்லைப் போலத்
தாக்கான சடந்தானு முலர்ந்து வற்றி
தவிக்குமே யடிக்கடிதான் கண்ணீர் தேடி
வாக்கான மதுரமொழி குளறிப் பேசி
வாய்வுதா னடிக்கடிக்கு மேலே நோக்கும்
நீங்கான மலசலமிதில் மாமிசங் காணும்
நேரான மாமிச மகோதரத்தி னேரே."

- யூகி முனி

There are various treatments available especially for *Yoniputru* in Siddha medicine.

3.2.2. Modern Aspect

Cancer

Cancer is a class of diseases characterized by out-of-control cell growth.There are over 200 different types of cancer and each is classified by the type of cell that is initially affected.

More dangerous or malignant tumors form by the following methods:

- a cancerous cell manages to move throughout the body using the blood or lymphatic systems, destroying healthy tissue in a process called invasion
- that cell manages to divide and grow, making new blood vessels to feed itself in a process called angiogenesis.

When a tumor successfully spreads to other parts of the body and grows, invading and destroying other healthy tissues, it is said to have metastasized. This process itself is called metastasis and the result in serious condition that is very difficult to treat.

According to the American Cancer Society, **Cancer** is the second most common cause of death in the US and accounts for nearly 1 in every 4 deaths. The World Health Organisation estimates that, worldwide, there were 14 million new cancer cases and 8.2 million cancer-related deaths in 2012.

Individual types of cancer

There are said to be over 200 different types of cancer. Some of the cancer types are as follows,

- Anal cancer
- Bladder cancer
- Bone cancer
- Breast cancer
- Cervical cancer
- Colon cancer
- Colorectal cancer
- Endometrial cancer
- Kidney cancer
- Leukemia
- Liver cancer
- Lymphoma
- Ovarian cancer
- Pancreatic cancer
- Prostate cancer
- Stomach cancer
- Testicular cancer
- Thyroid cancer
- Vaginal cancer
- Vulvar cancer

Causes of cancer

Genes - the DNA type

Cells can experience uncontrolled growth if there are mutations to DNA, and therefore, alterations to the genes involved in cell division.

Carcinogens

Carcinogens are a class of substances that are directly responsible for damaging DNA, promoting or aiding cancer. Some examples of carcinogens are,

- Tobacco
- Asbestos, Arsenic
- Radiation such as gamma and x-rays,
- The Sun rays
- Compounds in car exhaust fumes

When our bodies are exposed to carcinogens, free radicals are formed that try to steal electrons from other molecules in the body. Theses free radicals damage cells and affect their ability to function normally.

Other Medical Factors

As we age, there is an increase in the number of possible cancer-causing mutations in our DNA. This makes age an important risk factor for cancer. Several viruses have also been linked to cancer such as,

- Human Papillomavirus (a cause of cervical cancer)
- Hepatitis B and C (causes of liver cancer)
- Epstein-Barr virus (a cause of some childhood cancers)
- Human immunodeficiency virus (HIV)
- Anything else that suppresses or weakens the immune system - inhibits the body's ability to fight infections.
- Increases the chance of developing cancer.

Cancer classification:

There are five broad groups that are used to classify cancer.

- **Carcinomas** are characterized by cells that cover internal and external parts of the body such as lung, breast and colon cancer.
- **Sarcomas** are characterized by cells that are located in bone, cartilage, fat, connective tissue, muscle and other supportive tissues.
- **Lymphomas** are cancers that occurs or originates in the lymph nodes and immune system of tissues.
- **Leukemias** are cancers that begin in the bone marrow and often affects the blood cells.
- **Adenomas** are cancers that arise in the thyroid, the pituitary gland, the adrenal gland and other glandular tissues[29].

Cervical cancer

- Cervical cancer is the most common cancer in women aged 35 and below.

Cervical cancer is a type of cancer that occurs in the cells of the cervix. The cervix is the organ connecting the uterus and vagina.

It is usually a slow- growing cancer that may not have symptoms but can be found with regular pap tests. This is a procedure in which cells are scraped from the cervix and looked at under a microscope[30]. Cervical cancer is not thought to be hereditary. In 99.7% of cases, cervical cancers are caused by persistent infections with a virus called high-risk human papillomavirus (HPV).

HPV is a very common virus transmitted through skin to skin contact in the genital area. Around four out of five sexually active adults (80%) will be infected with some type of HPV in their lives. However, for the majority of women this will not result in cervical cancer. While HPV infection is common, cervical cancer is rare[31]. There are two types of cervical cancer . squamous cell carcinomas (80% to 90%) and adeno carcinomas (5% to 20%).

If the cancer has signs of both types, it is called mixed carcinoma. Early detection and treatment of cervical cancer is important to improve survival.

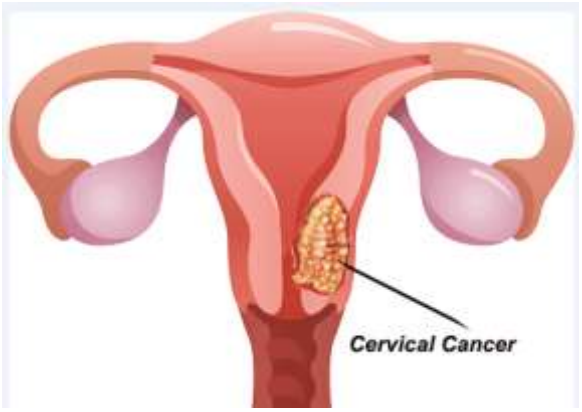


Fig.No.4. Shows Cervical cancer

History of Cervical Cancer[32]

Table No. 1.

Year/ Period	Key developments
19th century	Cervical cancer is identified as a sexually transmitted disease. At the end of the century, surgery is introduced for treating the disease.
Early 20th century	Epidemiologists discover that cervical cancer is common in female sex workers and also common in women whose husbands have a high number of sexual partners or were regular customers of prostitutes.
1920s	Papanikolaou develops his eponymous technique. The colposcope is developed.
1940s	Pap smear screening begins.
1980s	First concrete evidence that specific Human Papillomavirus (HPV) types are linked to cervical cancer. Tobacco use is linked to cervical cancer.
2000s	First Human papilloma virus (HPV) vaccine is released. Several nations introduce the vaccination, such as United States, Canada, Australia and Japan.
Recent years	Today, cervical cancer is both the fourth-most common cause of cancer and the fourth-most common cause of death from cancer in women. In 2012, approximately 528,000 cases of cervical cancer occurred, with 266,000 deaths.This is about 8% of the total cases and total deaths from cancer.About 70% of cervical cancers occur in developing countries.

Prevalence – Cervical Cancer

Cervical cancer has emerged as a second most common cause of cancer deaths among Indian women aged between 15 and 44 years, according to a report by Spain-based international public health institution Institute Catalàd'Oncologia (ICO) Information Centre.

On an average, **India** reports about 122,000 new cases of cervical cancer annually, with around 67,500 women succumbing to the disease, accounting for 11.1% of total deaths related to cancer.Cervical cancer comes next to breast cancer in terms of mortality rate in Indian women.

The two preventive strategies for cervical cancer include screening and vaccination. The report says just 3.1% women in India get screened, leaving a large population vulnerable to death from the disease.

According to internationally accepted protocol, all women of 25-64 years need to undergo screening every three years.If cervical abnormalities are detected at an early stage the abnormal tissue can be excised using day care by minimally invasive surgical procedures.

"Western countries, especially Europe, have taken cervical cancer very seriously even 40 years back and every women there get compulsorily screened; unfortunately in India it's not seen as a priority by the Government – although one-fourth of cervical cancer deaths occur in India," said Sharda Jain, gynaecologist and director at Global Institute of Gynecology, Delhi[(33)].

Predisposing Factors[(34)]

- Average age 35-45 years
- Coitus before the age of 18 years
- Multiple sexual partners
- Delivery of the first baby before the age of 20 years
- Multiparty with poor birth spacing between pregnancies.
- Poor personal hygiene
- Poor socioeconomic status
- Previously, exposure to smegma from uncircumcised partners was considered an important for lower incidence of cancer cervix amongst the Jews and Muslims.

- Smoking and alcohol
- Contraceptive pill- long term use of some common contraceptive pills slightly raises a women's risk.
- Long- term mental stress
- Women with STD, HIV infection, herpes simplex virus 2 infection, HPV infection (16, 18, 31, 33) or condylomata have a high predisposition to cancer.
- Immuno suppressed individuals (following transplant surgery)
- Women with pre invasive lesions
- Women who do not come for regular health check-up and pap test.

Human papillomavirus (HPV)

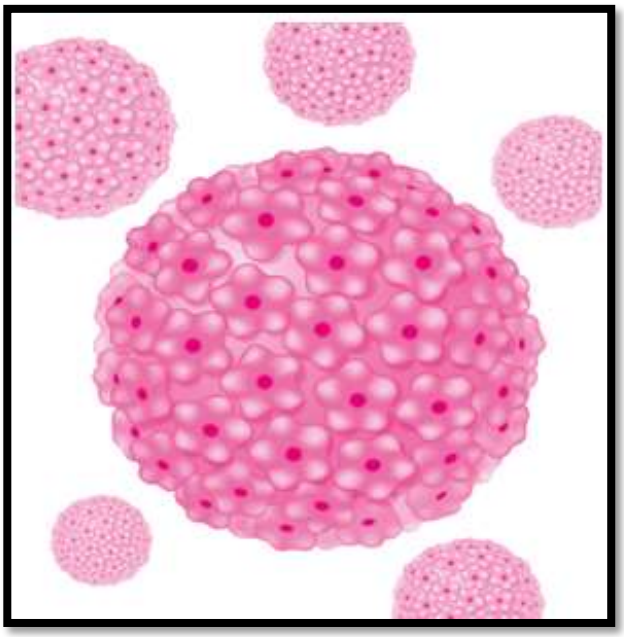


Fig .No. 5. Showing Human papilloma virus

Human papilloma virus (HPV) is an extremely common virus. HPV is the most widespread of all sexually transmitted viruses; four out of five (80%) of the world's population will contract some type of the virus once in their life.

If you catch HPV, in the majority of cases the body's immune system will clear or get rid of the virus without the need for further treatment.

There are over 100 identified types of HPV and each different type has been assigned a specific number. The majority of HPV types infect the skin on external areas of the body, including the hands and feet.For example, HPV types 1 and 2 cause verrucas on the feet. Different types affect different parts of the body, causing lesions.

- Around 40 of the HPV types affect the genital areas of men and women, including the skin of the penis, vulva (area outside the vagina), anus and the linings of the vagina, cervix and rectum .
- Around 20 of these types are thought to be associated with the development of cancer. The World Health Organization (WHO) International Association for Research on Cancer (IARC) defines 13 of these 20 types as oncogenic (cancer causing).

These high-risk types of HPV are

- HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. A person infected with a high-risk genital HPV will show no symptoms.
- In addition, there are nine HPV types that may also be associated with the development of cervical cancer, these are HPV 26, 53, 64, 65, 66, 67, 69, 70, 73 and 82.
- The remaining genital HPV types have been designated low risk as they do not cause cervical cancer but they can cause other problems, such as genital warts[(35)].

Pathology

Pap smear in invasive cancer shows tadpole cells, haemorrhage and necrosis in the background. It is customary to identify two types of cancers of the cervix. The first and more common variety is the epidermoid carcinoma. It arises from the stratified squamous epithelium of the cervix and accounts for almost 80% of all cancers in the cervix.

The second variety endocervical carcinoma arises from the mucous membrane of the endocervical canal, accounts for 20% of all cervical cancers.

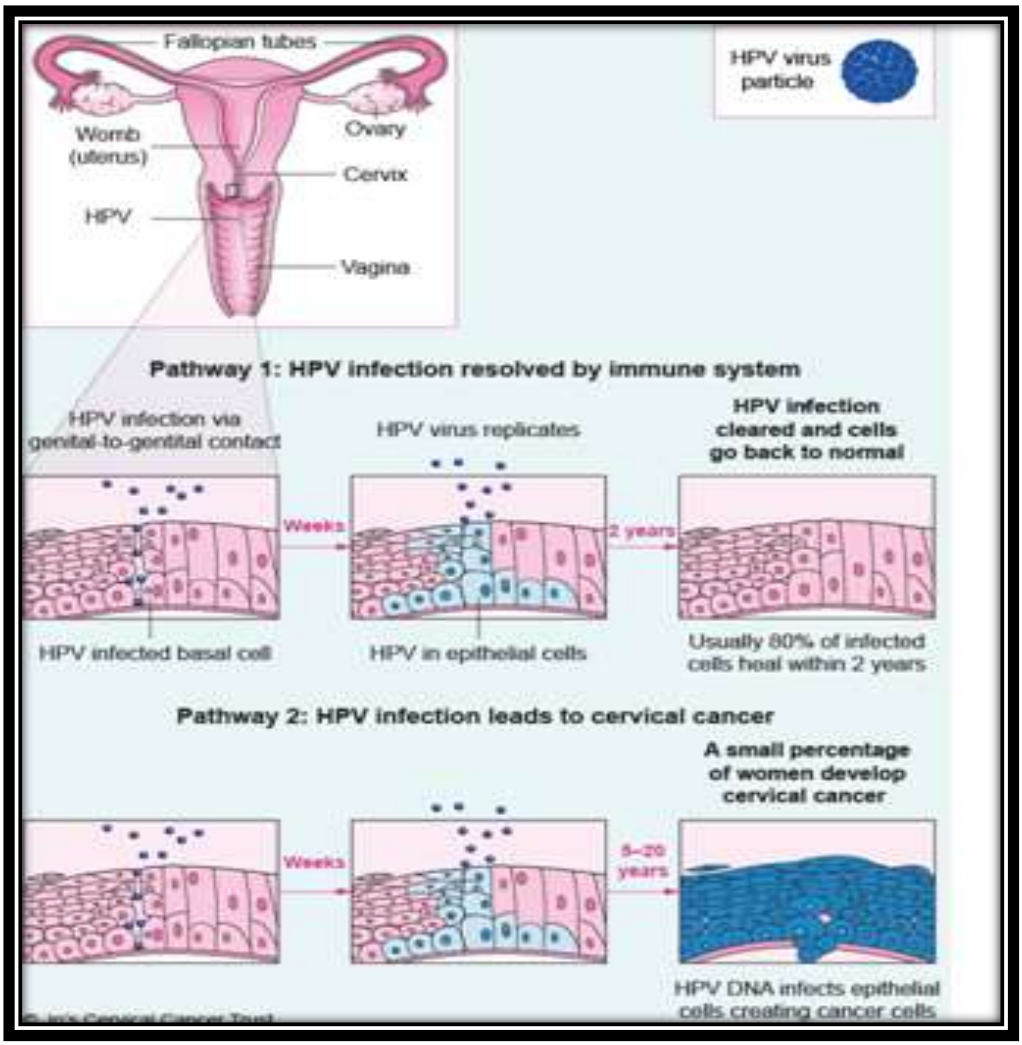


Fig.No. 6. HPV infection

Histologically, 95 percent of cervical cancers are squamous carcinomas and only 5 percent are adenocarcinoma. This is because the columnar epithelium of the endocervix often undergoes squamous metaplasia. Endocervical cancers of the cervix have recently increased in incidence because of prolonged use of oral combined contraceptive pills and progesterone's which have profound effect on glandular epithelium.

The malignant cells are endometroid, adenocarcinoma, clear cells and adenosquamous.

Squamous cell cancers of the ectocervix appear as proliferative growths, ulcers or as flat indurated areas. The common proliferative or cauliflower-like growth is vascular, friable and bleeds on touch.

It undergoes ulceration and necrosis, which is associated with an offensive foul smelling vaginal discharge. The leucorrhoeal discharge is often blood stained. Histologically, the tumor is graded as well-differentiated or ill-differentiated. The endocervical growth remains confined to the cervical canal for a long time causing a barrel-shaped enlargement of the cervix, and only at a late stage it protrudes beyond the external cervical os and becomes visible.

The mode of spread of the cancer is by continuity or by continuity, by lymphatic spread or through vascular embolism to distant sites like lungs, liver, bones, kidneys and brain. Ovarian metastasis occurs in only 1 percent.

Cervical cancer symptoms[36]

In the early stages of cervical cancer, a person may experience no symptoms at all. As a result, women should have regular cervical smear tests.

The most common symptoms of cervical cancer are:

- bleeding between periods
- bleeding after sexual intercourse
- bleeding in post-menopausal women
- discomfort during sexual intercourse
- smelly vaginal discharge
- vaginal discharge tinged with blood
- pelvic pain

Stages of Cervical cancer

Stage I

- Carcinoma strictly conflicted to the cervix IA Micro invasive carcinoma, not exceeding 5.0mm.
- IA1 Measured stromal invasion of less than 3.0mm in depth.
- IA2 Measured stromal invasion between 3 and 5mm in depth.
- IB Clinically visible lesion confined to the uterus
- IB1 Clinically visible lesion 4.0cm.
- IB2 Clinically visible lesion more than 4.0cm in dimension.

Stage II

- Cancer spread beyond the cervix, but not to pelvic wall or lower third of the vagina.
- IIA Tumor without parametrical invasion.
- IIB Tumor with parametrical invasion.

Stage III

- Tumor extends to the lateral pelvic wall, involves the lower third of vagina, and/or causes hydronephrosis or non functioning kidney.

Stage IV

- Tumor spread to the pelvic organs or distal metastasis.
- IVA Tumor involves bladder and rectum.
- IVB Widespread tumor with distant metastasis.

Differential Diagnosis:

- Tubercular ulcer
- Syphilitic ulcer
- Fibroid polyp
- Sarcoma of the cervix

Diagnosis [37]

The earlier cervical cancer is diagnosed, the more successful treatment can be done. Regular cervical screening can save thousands of lives every year.

- Cervical smear test
- HPV DNA Test

Additional tests including

- Biopsy
- Colposcopy
- Cone biopsy
- LLETZ
- Blood tests
- Examination under anesthesia (EUA)
- CT scan
- MRI
- Pelvic ultrasound

Treatments for Cervical cancer[38]

Surgery for Cervical cancer

Several types of 'surgery' can be used to help treat cervical cancer, although some of these destroy cervical tissue (with cold or with laser) rather than removing it.

- Cryo surgery
- Laser surgery
- Conization

Simple (total) hysterectomy:

This surgery removes the uterus (both the body of the uterus and the cervix) but not the structures next to the uterus (parametria and uterosacral ligaments). The vagina and pelvic lymph nodes are not removed. The ovaries and fallopian tubes are usually left in place unless there is another reason to remove them.

There are different ways to do a hysterectomy:

- Abdominal hysterectomy
- Vaginal hysterectomy
- Laparoscopic hysterectomy
- Laparoscopic-assisted vaginal hysterectomy
- Robotic-assisted surgery

Possible side effects:

- infertility (inability to have children).
- excessive bleeding,
- wound infection or damage to the urinary or intestinal systems.

Radiation therapy for Cervical Cancer[39]

Radiation therapy uses high energy x-rays or radioactive particles to kill cancer cells. Radiation therapy may be used for cervical cancer.

The two types of radiation therapy most often used to treat cervical cancer include:

1. External beam radiation
2. Brachytherapy

External beam radiation

One way to give radiation is to aim x-rays at the cancer from outside the body. This is called External beam radiation therapy **(EBRT).** Treatment is much like getting a regular x-ray, but the radiation dose is stronger.

Each radiation treatment lasts only a few minutes, but getting you into place for treatment usually takes longer. The procedure itself is painless.

When radiation is used as the main treatment for cervical cancer, EBRT is usually combined with chemotherapy (called concurrent chemoradiation). Often, a low dose of the chemo drug called cisplatin, but other chemo drugs can be used as well. The radiation treatments are given 5 days a week for a total 6 to 7 weeks. The chemotherapy is given at scheduled times during the radiation. The schedule is determined by which drug is used.EBRT can also be used by itself to treat areas of cancer spread or as the main treatment of cervical cancer in patients who can't tolerate chemoradiation.

Possible side effects of EBRT

Side effects of external beam radiation therapy for cervical cancer can include:

- Fatigue (tiredness)
- Upset stomach
- Diarrhea or loose stools (if radiation is given to the pelvis or abdomen)
- Nausea and vomiting
- Skin changes
- Radiation cystitis
- Vaginal pain
- Menstrual changes
- Low blood counts

Brachytherapy

Brachytherapy or internal radiation therapy, puts a source of radiation in or near the cancer. This type of radiation only travels a short distance. The type of brachytherapy used most often to treat cervical cancer is known as **intracavitary brachytherapy**. The radiation source is placed in a device in the vagina (and sometimes in the cervix). This is often used in addition to EBRT as a part of the main treatment for cervical cancer.

There are two types of brachytherapy:

- Low-dose rate (LDR) brachytherapy
- High-dose rate (HDR) brachytherapy

Possible short-term side effects of brachytherapy

Since the radiation only travels a short distance with brachytherapy, the main effects of the radiation are on the cervix and the walls of the vagina. The most common side effect is irritation of the vagina. It may become red and sore and there may be a discharge. The vulva may become irritated as well.

Brachytherapy can also cause many of the same side effects as EBRT, such as fatigue, diarrhea, nausea, irritation of the bladder and low blood counts.

Often brachytherapy is given right after external beam radiation (before the side effects can go away), so it can be hard to know which type of treatment is causing the side effect.

Long term side effects of radiation therapy

- Vaginal stenosis
- Vaginal dryness
- Weakened bones
- Swelling of the legs

Chemotherapy[40]

Chemotherapy (chemo) uses anti-cancer drugs that are injected into a vein or given by mouth. These drugs enter the bloodstream and can reach all areas of the body, making this treatment useful for killing cancer cells in most parts of the body. Chemo is often given in cycles, with each period of treatment followed by a recovery period. There are a few situations in which chemo may be recommended for cervical cancer.

As a part of the main treatment for cervical cancer

For some stages of cervical cancer, the preferred treatment is radiation and chemo given together (called concurrent chemoradiation). The chemo helps the radiation work better.

Options for concurrent chemoradiation include:

- Cisplatin given weekly during radiation. This drug is given into a vein (IV) about 4 hours before the radiation appointment.

- Cisplatin plus 5-fluorouracil (5-FU) given every 4 weeks during radiation.

Sometimes chemo is also given (without radiation) before and/or after chemoradiation.

Side effects of chemotherapy for cervical cancer:

Chemo drugs kill cancer cells but also damage some normal cells, which can lead to certain side effects. Side effects depend on the type and dose of the drugs and the length of time you are treated.

- Nausea and vomiting
- Loss of appetite
- Loss of hair
- Mouth sores
- Fatigue (tiredness)

Because chemotherapy can damage the blood-producing cells of the bone marrow. This can result in:

- An increased chance of infection (from a shortage of white blood cells)
- Bleeding or bruising after minor cuts or injuries (because of a shortage of blood platelets)
- Shortness of breath (due to low red blood cell counts)

When chemo is given with radiation, the side effects are often more severe. Nausea, fatigue and problems with low blood counts are often worse. Diarrhea can also be worse if chemo is given at the same time with radiation and the following side effects can occur,

- Menstrual changes
- Neuropathy
- Increased risk of leukemia

Other side effects are also possible. Some of these are more common with certain chemo drugs. Many side effects are short-term and go away after treatment is completed, but some can last for a longer time or even be permanent.

Targeted therapy / Immunotherapy for Cervical Cancer[41]

- Targeted therapy is a type of treatment that uses drugs or other substances to identify and attack specific cancer cells without harming normal cells.
- Monoclonal antibody therapy is a type of targeted therapy that uses antibodies made in the laboratory from a single type of immune system cell.
- Bevacizumab is a monoclonal antibody that binds to a protein called vascular endothelial growth factor (VEGF) and may prevent the growth of new blood vessels that tumors need to grow. Bevacizumab is used to treat cervical cancer that has metastasized (spread to other parts of the body) and recurrent cervical cancer.

The Vaccines[42]

There are currently two vaccines which protect against **HPV infection**, which are called Gardasil and Cervarix.

- **Gardasil**

produced by Merck, is designed to protect against four types of HPV

  - 16 and 18 (high risk for cervical cancer)
  - 6 and 11 (these types do not cause cervical cancer, but they do cause 90% of genital warts)

- **Cervarix**

produced by GlaxoSmithKline, is designed to protect against HPV types 16 and 18 only

Both vaccines are licensed in the UK. The NHS currently uses Gardasil to vaccinate girls.

- There is also a third vaccine called **Gardasil 9.**

  - This protects against high risk HPV types 16, 18, 31, 33, 45, 52 and 58, as well as HPV 6 and 11. Gardasil 9 has been approved for licensing by the Committee for Medicinal Products for Human use of the European Medicines Agency. This vaccine is likely to be given its licence by the European Commission later this year.

- The vaccination is free for all girls from the age of 11 in Scotland and 12 in the rest of the UK up to their 18th birthday, but only girls aged 11 to 13 in Scotland and 12 to 13 in the rest of the UK will be routinely offered the vaccine.
- The vaccination is given to girls at this age because their immune systems are at their strongest before puberty begins and the vaccination works best when the immune system is strong.
- HPV vaccination has been shown to prevent infection and abnormal cervical cell changes for at least 10 years, but modelling suggests it will last longer. Ongoing studies will show how much longer young women will be protected for and whether booster shots will be required.

Both Gardasil and Cervarix protect against the two highest risk HPV types. However, unfortunately, women can be infected with more than one type of HPV. Having the vaccine will provide protection against 70% of all cervical cancers and it will also prevent most of the more serious precancerous cervical changes (classed as moderate or severe cervical abnormalities) .

Prevention of cervical cancer[(43)]

There are a number of measures that can be taken to reduce the chances of developing cervical cancer.

- Human papillomavirus (HPV) vaccine
- Safe sex and cervical cancer
- Cervical screening
- Having fewer sexual partners
- Delaying first sexual intercourse
- Stopping smoking

3.3. Pharamacolgical Review

3.3.1. Siddha Aspect

Siddha system of medicine gave us so many drugs for different type of cancers. Some important medicines are mentioned below,

Pills:

- Chithiramoolaakuligai[44]
- Maha Kodasoozhi Mathirai
- Aswaganthathi vadagam

Chooranam

- Vallathy Chooranam
- Megaroga Chooranam
- Kukkilathy Chooranam
- Garudakodi Chooranam
- Sorkkamara Chooranam
- Thumbai Chooranam
- Vembadam pattai Chooranam
- Karanthai Chooranam[45]

Parpam:

- Thambira Parpam[46]
- Gandhaga Poora Parpam[47]
- Kariya Parpam
- Sootha Parpam
- Rasa Parpam
- Naga Parpam
- Thalaga Parpam
- Sandarasa Parpam

Chendhooram:

- Panchapadana Chendhooram[48]
- Swaranapushpa Rasa Chendhooram[49]
- Muthu Chendhooram[50]
- Kandhaga Chendhooram
- Gowri Chinthamani Chendhooram
- Linga Chendhooram
- Rasa Chendhooram
- Thambira Chendhooram

- Pavala Vanga Chendhooram
- Kalamega Narayana Chendhooram
- Kalameganarayana Chendhooram[51]
- Ashtapairava Chendhooram
- Sandamarutha Chendhooram
- Navachara Chendhooram
- Naga Chendhooram[52]
- Namatchivaya Chendhooram[53]
- Narayana Chendhooram[54]

Nei:

- Chitramoola Nei[55]
- Kukkil Nei
- Thengai Nei
- Vallarai Nei

Ennai:

- Perungaya Ennai[56]
- Singi Thylam
- Mega Santhanathy Thylam
- Chinthamani Ennai
- Meganathi Ennai
- Visha Rajanga Thylam
- Pachai Thylam
- Vippuruthi Ennai
- Sengathari Thylam
- Mega Rasanga Ennai
- Puda Thylam
- Gandhaga Thylam[57]
- Sengottai Thylam

Mezhugu:

- Rasagandhi Mezhugu[58]

- Kanaga Linga Mezhugu
- Guru Sanjeevi Mezhugu
- Vaalai Rasa Mezhugu
- Gandhaga Mezhugu[59]
- Korasanai Mezhugu
- Veera Mezhugu

Kattu:

- Poorakattu[60]

Pathangam:

- Lingapathangam[61]
- Gurupathangam
- Putru pathangam
- Veera Rasa pathangam

Others :

- Rana Pugai
- Veelai seelai
- Kulirntha pachai
- Chithiravallathi Legium

Siddha drugs for Yoniputru

Pills :

- Chitramoola Kuligai

Chooranam:

- Karanthai chooranam

Parpam:

- Thambira Parpam

- Velli Parpam
- Gandhaga Parpam
- Karuvanga Paarpam

Chendhooram:

- Muthu Chendhooram
- Namachivaya Chendhooram
- Swarnapushpa Rasa Chendhooram
- Panchapadana Chendhooram

Thylam:

- Chitramoola Nei
- Vallarai Nei
- Megathu Ennai
- Gandhaga Thylam
- Sathrusangara Ennai
- Perungaya Ennai
- Meganathi Thylam

Mezhugu:

- Vithu Rasa Meazhugu
- Korosanai Mezhugu
- Rasaganthi Mezhugu
- Gandhaga Mezhugu
- Amirtha Nandhi Mezhugu

Kattu:

- Poora Kattu

Pathangam:

- Linga pathangam

Kirutham:

- Vallarai kirutham

Others:

- Gandha Rasa Villai
- Megam pokkum Rasagandhi
- Soolai Kudori

3.3.2. Modern aspect

Anti-cancer drugs

The drugs which prevent neoplasm are known as anti-cancer drugs. They also called Anti-neoplastic drugs.

Purpose

Anti –cancer drugs are used to control the growth of cancerous cells. Cancer is commonly defined as the uncontrolled growth of cells, with loss of differential and commonly, with metatasis, spread of the cancer to other tissues and organs.

Cancers aremalignant growths. In contrast , benign groths remain encapsulated and grow within a well-defined area.

Drug therapy is used when the tumor has spread or may spread, to all areas of the body.

They may be divided into two classes

Cycle specific

Cycle specific drugs act only at specific points of the cell's duplication cycle, such as anaphase or metaphase.

Non cyclic specific

Drugs may act any point in the cell cycle, in order to gain maximum effect, anti-neoplastic drugs are commonly used in combinations.

Precautions:

Because anti neoplastic agents do not target specific cell types, they have a number of common adverse side effects.

- Hair loss is common due to the effects on hair follicles
- Anaemia
- Immune system impairment
- Clotting problem are caused by destruction of the blood forming organs, leading to reduction in the number of red cells, white cells and platelets.
- Bone marrow depression

Interactions:

Anti- cancer drugs may interact with a number of other medicines. When this happens, the effects of one or both of the drugs may change or the risk of side effects may be greater.

Clasification of Anti-cancer drugs[62]

1. Alkylating agents
2. Antimetabolites
3. Natural products
4. Miscellaneous
5. Hormones and Antagonist

Alkylating agents

- Cyclophoshamide
- Ifosfamide
- Triazene
- Methyl hydrazines
- Alkylsulfonates
- Cisplatin
- Carboplatin
- Oxaliplatin
- Nitrosoureas
- Melphalan

Antimetabolites

- Methotrexate
- Purine antagonists
- Pyrimidine antagonists- 5-fluorouracil

Natural products

Plant products

- Vincristine
- Vinblastin
- Taxanes- Paclitaxel
- Etoposide

Micro-organism products

- Antibiotics- Doxorubicin, Bleomycin
- L-Asparaginase

Miscellaneous

- Hydroxyurea
- Imatinib Mesylate
- Leucovorin
- Geftinib
- Pamidronate
- Gemcitabin

Hormones & Antagonists

- Dexamethasone
- Prednisone
- Ethinyloestradiol
- Megestrol acetate
- Flutamide
- Letrozole
- Growth hormone
- Glucagon

Table No.2. Anti cancer drugs[63]

Generic (Brand Name)	Clinical Uses	Common side effects to Drug
Altretamine(Hexalen)	Treatment of advanced ovarian cancer	Bone marrow depression, nausea, and vomiting.
Asparaginase (Elsper)	Commonly used in combination with other drugs; refractory acte lymphocytic leukemia	Liver, Kidney, pancreas, CNS abnormalities.
Carboplatin (Paraplatin)	Palliation of ovarian cancer	Bone marrow depression, nausea, and vomiting.
Clorambucil (Leukeran)	Chronic lymphocytic leukemia, non Hodgkin's lymphomas, breast and ovarian cancer.	Bone marrow depression, excess uric acid in blood.
Bleomycin (Blenoxane)	Lymphomas, Hodgkin's disease, testicular cancer.	Hair loss, stomatitis, pulmonary toxicity,hyper pigmentation of skin.
Busulfan (Myleran)	Chronic granulocytic leukemia	Bone marrow depression, pulmonary toxicity.
Carmustine	Hodgkin's disease, brain tumors,, multiplemyeloma, malignant melanoma.	Bone marrow depression and vomiting.
Cisplatin (Platinol)	Treatment of bladder, ovarian,uterine, testicular, head and neck cancer.	Renal toxicity and ototoxicity.
Cyclophosphamide (Ctoxan)	Hodgkin's disease, non Hodgkins lymphoma, neroplastoma. Often used with other drugs for breast, ovarian and lung cancer; acute lymphoplastic leukemia in children.	Bone marrow depression, nausea, vomiting, hair loss, inflammation of the bladder.

Generic (Brand Name)	Clinical Uses	Common side effects to Drug
Ethinyl estradiol (Estinyl)	Advanced breast cancer in post menopausal women, prostate cancer.	Excessive calcium in blood, anorexia, oedema, nausea, vomiting; feminizing effects in men.
Mitotane (Lysodren)	Cancer of the adrenal cortex (inoperable)	Damage to adrenal cortex,nausea, anorexia.
Paclitaxel (Taxol)	Adavanced ovarian cancer	Bone marrow depression, hair loss, nausea,
Mitomycin (Mutamycin)	Bladder, breast, colon, lung, pancreas, rectum cancer.	Bone marrow depression, nausea and vomiting.
Tamoxifen (Nolvadex)	Advanced breast cancer in post menopausal women.	Nausea, vomiting , ocular toxicity, hot flashes.

Pharmacological review of Cancer:

The pharmacological screening of plants, minerals and animals is an essential mean for the invention of new, harmless and effective drugs. Over 50,000 plants have therapeutic virtues in the world and around 80% of human use medicines based on plants and salts at least once in their life.

Medicinal plants and mineral share diversified chemical constituents which are important for the discovery of new active molecules against many types of Cancer. Active compounds from many medicinal plants and minerals with effective cytotoxic properties were developed into Anti-Cancer drugs.

Nowadays it has become mandatory to monitor the quality of life of patients while in treatment of Cancer. There should be health awareness in the quality of life of Cancer patients treated with chemotherapeutic drugs because they are very much affected even for a long time after withdrawal of drugs.

Therefore, the challenging task at this moment is to identify the quick and novel methods that can identify and develop molecules, which can be of therapeutic value in human Cancers.

This urgently necessitates screening of a large number of compounds. For this purpose both, the *in-vitro* and *in-vivo* models are employed for systematic screening of an Anti-Cancer drug.

In-Vitro method

In in-vitro cytotoxicity studies on cell line various cell staining methods are used in order to indirectly estimate the number of viable cells present after treatment. Ideal test in assessing cell proliferation and cytotoxicity should have as main feature in-vitro be simple fast, efficient economical, reproducible, sensitive, safe, effectiveness far viable cell population and do not show interference with to evaluate the compound.

Advantages[64] **:**

- Reduce the usage of animals
- Testing the ability of the compound to kill the cell by taking the advantage of various properties of cell.
- Able to process the large number of compounds quickly with minimum of quantity
- Range of concentration used is comparable to that expected for invivo studies

Disadvantages:

- Difficulty in maintaining of culture
- Show negative result for the compounds which gets activated after metabolism and vice versa
- Impossible to as certain the pharmacokinetics

How to culture cell line?

- Tumor cell line derived from several cancer types
- Adaptable to a suitable growth medium
- Show reproducible profile for growth and drug sensitivity

- The lives were prepared and preserved using regents such as DMSO during freezing.
- Thawing – bringing the freezed ampoule to room temperature by slow agitation.

Cell lines for Cancer:

There are plenty of cell lines are available for research purpose. Only very few are listed.

Table No.3. Cancer cell lines

S.No	Cell Name	Tissue	Species
1.	UM-UC	Bladder	Human
2.	FM3A	Breast	Mouse
3.	C170	Colon	Human
4.	SHP77	Lung	Human
5.	RAG	Kidney	Mouse
6.	HF 1	Liver	Rat
7.	MEWO	Skin	Human
8.	TT	Thyroid	Human
9.	OV	Ovary	Human
10.	C 6	Neural (Gilial tumor)	Rat

Assay[65]

For energy metabolism and Autophagy:

- FAD assay
- ATP assay
- Lysosome detection`

For nuclear signaling, DNA damage and cell proliferation

- P^{53} assay
- Topoisomerase II assay
- P^{21} assay
- Cell proliferation assay

- Mdm 2 assay

For Inflammation, Angiogenesis and Metastasis

- Cytokine and chemokine assay
- STAT 1,2,3,6 assay
- COX-2 activity assay
- LDL uptake assay

For Apoptosis, Pyroptosis and Necrosis

- Caspase 1 assay
- Bax assay
- Cytolysis assay
- Calpanin assay

For cancer signaling pathway and phenotype

- ERK assay
- c-AMP assay
- c-Jun test

In -Vivo Models

Many animal species develop cancers spontaneously and are valuable for understanding the biology of sporadic cancer development in humans. The major use of spontaneous cancer models is to compare the biology with human, in these animals are increasingly valuable for cross – comparison of response or resistance to clinical agents used for patients[(66)].

Animal models

1. Mouse Cancer models

I .GEM – Genetically engineered mouse model

II.Inbred mice (systematic sibling mating)

III.Transplantation models

- Allograft models (syngeneric tumor tissue derived from same genetic mouse)
- Xenograft models (actual human cancer cells or solid tumors are transplanted into host mouse)

IV. Carcinogen induced and spontaneous models

- Digestive system cancer induced by polycyclic aromatic hydrocarbons
- Chemically cancer induced by Cadmium and Arsenic
- Radiation-skin cancer by Ultra violet radiation: leukemic changes by ionizing radiation.

2. Rat cancer models

I .Genetically altered rats

- Treat embryos with DNA damage causing chemical mutagen. Frequently N-ethyl-N – nitrosourea (ENU) is used.
- Insertion of mutagenesis strategies (Retro virues)
- Transgenic strategies (Pro nuclear injection of DNA) – quickly developed and more effective models.

II. Inbred rats

3. Other laboratory animal models

- Hamster
- Rabbits
- Zebra fish

4. Other animal models

- Dogs
- Cats
- Goats
- Horses
- Pigs

There is also work done with various species, such as Baboons, Chimpanzees, Macaques, Marmosets and Tamarins.

Cerviacl cancer cell lines

- HeLa (HPV 16)
- SiHa (HPV 18)
- C 33A (HPV Negative)
- CaSki

Induction of cervical cancer in animal models:

- Cervical neoplasia is induced in mouse by an extract of varicella zoster virus infected cells (HPV or Herpes simplex virus type 2 DNA)
- Genomic HSV 2 DNA was isolated from infected HE p^2 cells and separated from host cell DNA by Cesium chloride density gradient centrifugation.
- The DNA was applied to mouse cervix for period of 80 to 100 weeks. Should be examined monthly to detect abnormalities.

3.4. Pharmaceuctical Review

Parpam[67] [68]

Concept and terminology:

Parpam is equivalent to calx, which is prepared by a process of calcinations. There are rare exceptions to this general concept, where no heating at all is required in the process, Muthuparpam is an example where, according to certain texts, the pearls are simply ground with rose water in to a fine powder . Kungiliya Parpam is an example where the drug sal- dammar is not actually calcined, but is heated to melting stage with coconut water and then dried and powdered.

The term *Parpam* is apparently a tamilized form of the Sanskrit word bhasma. The correct tamil translation would be "*Neeru*"(நீறு) which would mean an ash."*Saambal*" (சாம்பல்) is another word equivalent to an ash or calx. However, term *Parpam* has held the ground in siddha medicine.

Parpam are made from the given drugs according to specified methods. The drugs taken may either be organic (as in the case of 'Kungiliya Parpam', 'Aamaioduparpam', or 'Peranda Parpam') or inorganic (as in the case of 'Kalnaar Parpam', 'Thanga Parpam', 'Velli Parpam') in origin. Accordingly, these may be bones or horns , shells or secretions and metals or minerals or ores or salts. Chemically, most of the drugs taken are oxidized when they attain the '*Parpam'* from barring a few. Physically the *Parpam* is composed of particles in a fine state of division, with a major portion in the colloidal dimensions. This ultra fineness is obtained by efficient oxidation and trituration.

Physiologically, the particle fineness of great importance. Most compounds of metals and minerals are not absorbed by the body from the digestive tract, because

under ordinary circumstances, these substances could not be reacted upon by the secretions of the digestive system, so as to render them absorbable by the organism. This difficulty is overcome when the individual particles of this compound are very minute.

For instance, Mica is practically insoluble in water or dilute hydrochloric acid. But, when it is prepared in to a *Parpam* and is highly comminuted, a considerable portion goes in to solution in hydrochloric acid approximately of the same strength of gastric juice.

Similarly, metallic gold produces, toxic symptoms simulating those of Arsenic, but in the colloidal state, it has been found to be beneficial to the system. This shows the usage of Thanga parpam as an efficient vitalizer in the Siddha system from time immemorial.

The repeated grinding and heat treatment given to the materials, reduces the materials so treated, into different chemical compounds or at least into fine particles, which are capable of being acted upon by the system and absorbed and incorporated into the tissues.

Preparation

Equipments required:

- Mortar and pestle
- Vessels and spoons to handle liquids
- Long ribbons of tough cloth and fine clay.
- Pairs of shallow earthen discs of identical dimensions
- Well dried cow dung cakes in sufficient numbers.
- Fine cloth pieces for filtering juices and decoctions.
- Spatula for handling powders.

Notes on the drugs used:

- The drugs taken should be clean and those that should be purified should necessarily be taken through the process of purification according to the convention of Siddha science
- The materials taken should be properly identified.

- Great care should be exercised during the process of handling molten metals and pouring them into specified juices, so as to avoid accidents

Method of preparation:

The drugs are ground according to the particular recipe, with other drugs, juices or decoctions and the resultant mass is made into small, thin circular cakes and dried. When they are well dried, they are taken for calcinations.

The materials ready for calcinations, is put into an earthen disc described earlier and covered by inverting another disc and sealing the rim with the cloth ribbon one side of which is smeared with wet clay. This makes a capsule type crucible. When the seal is dry, the capsule is placed in the kiln for calcinations.

The Kiln or Pudam as it is called in Tamil, is made by digging a pit of appropriate dimensions in the soil and filling it with the recommended number of cow dung cakes, which is the fuel. It is better that the interior of the pit is lined with bricks, so that the pit could be used repeatedly.

Seventy five percent of the recommended numbers of dung cakes are arranged in the pit and then the capsule (or capsules) is placed in the center. The rest of the dung cakes are arranged above this and the top is somewhat dome shaped. When some burning charcoal pieces are placed on the dome, the dung cake below them catch fire and the fire spreads all around in an uniform manner.

The kiln will burn for a long time, until all the dung cakes are burnt and converted in to ashes. When the kiln cools down, the ashes are very carefully removed and the capsule is taken out without damaging the seal. The exterior of the capsule is thoroughly brushed to remove the ashes and the seal is scraped off and removed.

The content of the capsule are recovered and the remnants that adhere to the walls are gathered by gentle scraping and brushing.

For the complete transformation of the material into the *Parpam* state , the process of grinding , drying and calcinations may have to be repeated several times or at least as many times as directed in the recipe. However, the calcinations is repeated until a satisfactory product is obtained. But in those instances where the number of

calcinations is definitely indicated, the process should be repeated accordingly, even if a satisfactory *Parpam* is obtained within a few calcinations.

While preparing the *Parpam* of Lead, Tin and Zinc the number of dung cakes used as fuel , should always be comparatively lesser than the number used for other metals, because excessive heating will result in the reversion of the *Parpam* to the metallic state.

Test for complete calcinations

The primary factor being the color of the final product, there are the following tests to find whether the given material has been satisfactorily calcined and comminuted.

- As indicated earlier, the color shades attained by different materials when they are calcined are of prime importance and this is learnt mostly by experience.
- A pinch of the *Parpam* gently placed on the still surface of water in a vessel, does not sink immediately
- When a pinch of the *Parpam* is taken and pressed between the fingers, the mass shows the finger prints and the particles enter the fine folds of the finger, if calcinations is satisfactory.
- The *Parpam*, if satisfactorily completed, is irreversible to its metallic state when heated with a mixture of cane jaggery, hemp powder ,bdellium , ghee and honey

Storage of *Parpam*:

Parpams are usually stored in glass bottles. For smaller packings, vials of glass are used. Encapsulation could also be done when equipment is available. It is highly desirable that preparations be stored and retained in relevantly labeled containers. They are said to retain their potency for 100 years if properly stored.

3.5. Lateral research

Urginea indica

- *U. indica*, an anti-proliferative assay with ER positive breast cancer cell line (MCF-7) and ER negative **Breast cancer** cell line (BT-549) was performed. The aqueous bulb extract of red variety exhibited 82% of anti-proliferative

activity against MCF-7 compared to control, whereas white variety inhibited 62%.

- The percent of inhibition of DPPH radical was 16.5, 18.0, 14.0, 11.5% with aqueous extracts of bulb, leaf, stem and root of white variety respectively,whereas 18, 18, 16, 14% respectively, with red variety at 25 mg/ml. It has **Anti-oxidant** property[69].
- The extract of the bulb of *Urginea indica Kunth*. were collected by using of alcoholic extraction. The **anti-inflammatory** action of the alcoholic Extract of the bulb of the plant *Urginea indica* was evaluated in rats (female) against carrageenan induced edema i.e., using plethysmographic method[70]
- The crude aqueous-methanol extract of *Urgineaindica* bulb (Ui.Cr) was tested on mice and isolated gut preparations. *Urgineaindica*, which was tested positive for alkaloids, tannins and coumarins, increased faecal output and accelerated charcoal meal transit in mice (6–12 mg/kg, p.o.) This data, indicating the presence of a **gastrointestinal stimulant effect** in *Urginea indica* possibly mediated through a cholinergic mechanism, provide a rationale for the use of *Urginea indica* in indigestion and constipation[71].

Sulfur:

- The growth inhibitory and apoptosis-related effects of a newly developed highly purified sulfur (HPS) on immortalized human oral keratinocytes (IHOKs) and on oral cancer cells representing two stages of oral cancer (HN4, HN12) based on a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, Western blotting, cell cycle analysis, and nuclear staining[72].
- The both diol-containing compounds, 2a and 3, were the most cytotoxic of the sulfide series against V-79 cells in vitro (IC(90) = 2.1 microM and 1.9 microM, respectively). A preliminary **anticancer s**creening against **P388 leukemia** showed that 2a is highly active in vivo as well[73].
- Allysulfur compounds from garlic are reported to reduce the incidence of Breast, Colon, Skin, Uterine and Lung cancers and to depress proliferation of tumor cells.
- Allysulfur compounds contributes cell proliferation, although the suppression of Anti-cancer activity [74].

Mercury

- The drug Arkashara Rasa showed potent activity against pancreatic cancer cells (MIA-PaCa-2). LDH activity confirmed that AR was active against **Pancreatic Cancer**cells [75].

- The incineration process of theMercury and Sulfur macro particles are became very smaller and this may be possible for devoid of toxicity and more potent in **Anticancer** therapy.
- The results acquired from the *in-vitro* studies achieved via the ***HeLa* cell** lines reveals that theunique Siddha medicine Gowri *Chinthamani Chendhooram* (contains Mercury and Sulfur) have a potent **Anticancer activity** [76].

4. MATERIALS AND METHODS

4.1. Preparation of the Drug

Selection of Drug

"***Rasaparpam***" is one of the Herbo mineral Siddha formulation which was indicated in the Siddha Sastric literature "***Aathmarakchamirtham Ennum Vaithiya Saara Sangiragam"*** written by **Kandhasamy Mudhaliyar**[77]. Hence the present study is taken to validate the scientific background behind this drug through the evaluation of toxicological, pharmacological and elucidation of structural components of the formulation ***Rasaparpam***.

Ingredients

1. *Vaalai Rasam* (Purified Elemental Mercury) 35gms
2. *Gandhagam* (Sulphur) 35gms
3. *Kattuulli* – Indian squill (*Urginea indica*) 35gms

Collection of crude drug:

- Sulphur, Red sulphide of Mercury and *Plumbago indica* were bought from R.N.Rajan Country raw drug shop at Parrys corner in Chennai, Tamilnadu.
- The *Kattuulli (Urginea indica*) collected from the Kolli hills, Namakkal District.

Identification and Authentication:

- The raw materials were identified and authenticated by Botanist and Gunapadam experts, Government Siddha Medical College, Arumbakkam, Chennai. A specimen sample of each raw material has been kept in the department for future reference. (Reg.No:GSMCC/PGGM/0001-0003/14-17)

4.1.1. Purification of raw drug

All the raw materials were purified as per the Siddha literature[78]

1.*Vaalairasam* (Processed Elemental Mercury)

Kodiveli (Plumbaco zeylanica) roots were grounded well into a paste and smeared inside a mud pot and dried well. 7 *palam* (245gm) *Lingam (Cinnabar)* was placed in another mud pot and the mouth was covered with *Kodiveli* smeared pot. Then their mouths were sealed with mud pasted cloth. This apparatus was mounted in the earthen stove and burnt for 3 hours using fire wood. Purified elemental Mercury was obtained in the upper pot. Finally collected and used for the preparation of *Rasaparpam.*

2. Sulphur

Sulphur is placed in an Iron spoon. A small quantity of cow's butter is added and the spoon is heated till the butter melts, this mixture is immersed in inclined position in cow's milk. This procedure is repeated for 7 times to get purified Sulphur. Each time fresh milk is to be used.

Sulphur mixed with ghee and heated. After it starts melting, pour into the milk. Repeat the procedure for 7 times to get purified Sulphur.Each time fresh milk is to be used.

***3. Kattuulli* - (*Urginea indica*)**

The dust particles were removed.

4.1.2. Preparation of the trial drug – *Rasaparpam*

Procedure

- *Indian squaill* and Sulphur – each 35gms were taken and placed in a stone mortar and ground well to get a paste. This is made as a pellet.
- The pellet was kept in an earthen pot and medicated oil was obtained by calcination method using the equipment – *Kuzhi puda karuvi*
- This oil got by *Pudam* (*Kuzhi puda thylam*) was added to *Vaalai Rasam* and kept exposed to sun light for one day. And the substance was dried.

- This was ground with the above oil and made as a pellet.
- Bricks were taken and crushed into pieces to the size of betel nut.
- Half of the brick pieces were spread in a round bottom earthen pot. 1 *padi* (1.3lit) of salt was layered above the brick pieces and the pellet was kept over the salt.
- The pot was covered with earthen dish and sealed with 8 layers of mud pasted cloth and heated using fire woods through high flame (*Kaadakkini*).
- After that the covering dish was removed. The sublimate was obtained in the upper earthen dish. Finally *Parpam* was collected and ground well.
- The *Rasaparpam*was collected and kept in an air tight container.
- The *Rasaparpam* was labelled as RP.

Route of Administration:

Oral

Dosage:

Panavedai alavu (488 mg)

Adjuvant:

Palm jaggery

Indication:

Tumor**, Cervical cancer**, Inguinal bubo, Abscess.

Analytical study as per Siddha literature

Siddhars explained many testing procedures and standardization methods for different kind of medicines. Under this chapter the colour, heaviness, appearance and sense on touch and other test are discussed. For a Siddha formulation these characters are required for standardization.

1. Colour:

Parpams are white in colour except Gold *Parpam* which is yellow colour.

RP was taken and watch the colour is observed in day light by naked eyes.

This is displayed in Table No:7

2. Floating on Water:

This procedure is conducted to know about the density of the RP whether it is heavier or lesser than water.

A pinch of *Parpam* gently placed on the still surface of water in a vessel, did not sink immediately. It was found that the *Rasaparpam* particles floated over the surface of water indicated lightness of the trial drug.

3. Lines on fingers:

Parpam in well prepared form should be as fine powder. When taken between thumb and index finger, the fine powder will fill up the lines of the finger print. A pinch of RPwas taken in between the thumb and index finger and rubbed. It was found that the RP entered into the lines of the finger and was not easily washed out from the lines, confirmed its fineness.

4. Irreversible reaction:

The well prepared *Parpam* does not get reversible to its metallic state when heated with a mixture of cane jaggery, hemp powder, ghee and honey. A pinch of RP was taken and mixed with cane jaggery, ghee and honey. It was observed that RP did not reverse to its metallic state.

5. Tastelessness:

The well prepared *Parpam* should be completely tasteless. Presenceof any taste like sweet or bitter indicate incomplete preparation which needed another Calcination process. When a small amount of RP was kept on the tip of the tongue, no specific taste was found.

6. Lustreless:

If any shining particle is present in *Parpam,*it indicates that the *Parpam* is not manufactured properly and contains unchanged substances like minerals, metals and other toxic substances. There should be no shining particles present in the well

manufactured *Parpam*. The RPwas taken in a petri bowl and observed for any lustre in daylight through magnifying glass. No lustre was observed in the *Parpam*.

The results were tabulated in Table no:7

4.2. Standardisation of the Drug

Standardisation of drug means confirmation of its identity, determination of its quality and purity; detection of nature of adulterant by various parameters like morphological, microscopical, physical, chemical and biological evaluations[79].

Standardization of the drug *Rasaparpam*:

Standardization of drugs helps to prove its identity and determination of its quality and potency. Standardization of the Herbo-mineral formulation is based on the qualitative and quantitative analysis through Physico-chemical investigations and instrumental analysis. The Physico-chemical analysis of the prepared Herbo-mineral drug have been done at Central Research Institute, Arumbakkam, Chennai and elemental analysis have been done at IIT, Chennai. (FTIR, SEM, ICP-OES, RAMAN Spectroscopy)

4.2.1. Physico chemical analysis:

Physico chemical studies of the trial drug have been done according to the WHO guidelines.

pH value:

Potentiometrically pH value was determined by a glass electrode and a suitable pH meter.

Loss on Drying:

Loss of weight expressed as percentage resulting from water by volatile matter of any kind that can be driven off under specified conditions. The test is carried out on a well mixed sample of the substance, if the substance is in the form of large crystals, reduce the size by rapid crushing to a powder form.

The powdered drug was dried in the oven at 100- 105°C to constant weight.

Action on heat:

A small amount of the RP was taken in a dry test tube and heated gently. If strong white fumes evolve indicate the presence of Carbonate.

Flame test:

A small amount of the RP was made into a paste with con. HCl in a watch glass and introduced into non-luminous part of the Bunsen flame. Appearance of bluish green flame indicates the presence of Copper.

Ash Test:

A filter paper is soaked into a mixture of RP and Cobalt nitrate solution and introduced into the Bunsen flame and ignited. Appearance of yellow colour flame indicates the presence of Sodium.

Determination of Total Ash

The residue remaining after incineration is the ash content of the drug.

Total Ash Value:

Total ash method is used to measure the total amount of material remaining after incineration[80].

Acid Soluble Ash:

It is the residue obtained after boiling the total ash with dilute HCl and igniting the remaining insoluble matter.

Water Soluble Ash:

It is the difference in weight between total ash and residue after treatment of total ash with water.

4.2.2. Preliminary Basic and Acid Radical studies

Preparation of extract[81]

5gm of RP was weighed accurately and placed in a 250ml clean beaker and added with 50ml of distilled water. Then it was boiled well for about 20 minutes. Then it was cooled and filtered in a 1000ml volumetric flask and made up to 100ml distilled water.

Table.No.4. Test for basic radicals

PROCEDURE	OBSERVATION	INFERENCE
Test for Potassium: A pinch of sample is treated with 2ml of sodium nitrate solution and then treated with 2ml of cobalt nitrate in 30% of glacial acetic acid.	Formation of Yellow colour precipitate	Presence of Potassium
Test for Calcium: Taken2 ml of extract in a clean test tube. Then acetic acid and potassium chromate solution were added	No Yellow precipitate	Presence of Calcium
Test for Magnesium: 2ml of extract was taken in a clean test tube, few drops of Magnason reagentwas added in drops.	Formation of Blue colour precipitate	Presence of Magnesium
Test for Ammonium: 2mlof extract was taken in a test tube and added few ml of Nessler's reagent.	Appearance of Brown colour	Presence of Ammonium

PROCEDURE	OBSERVATION	INFERENCE
Test for Sodium: 2 pinches of *Rasaparpam* was mixed with HCl and made it into paste. And introduced into the blue flame of Bunsen burner.	Appearance of intense Yellow colour	Presence of Sodium
Test for Iron (Ferrous): 2ml of extract was taken in a clean dried test tube and conc. HNO_3 and ammonium thiocyanate were added.	Appearance of Blood red colour	Presence of Ferrous iron
Test for Zinc: 2 ml of the extract was taken in a test tube and Potassium ferro cyanide solution was added.	Formation of White colour precipitate	Presence of Zinc
Test for Aluminium: To the 2m1of the extract was taken in a test tube sodium hydroxide drops were added to it.	White precipitate obtained	Presence of Aluminium
Test for Lead: 2 ml of extract was taken in a test tube and added with 2ml of potassium iodide solution	Formation of yellow colour precipitate	Presence of Lead
Test for Copper: To a small portion of aextract dilute hydrochloric acid was added and then hydrogen sulphide gas is passed through the solution.	Black precipitate	Presence of Copper
Test for Mercury: 2m1 of the extract wastaken in a test tube and treated With 2ml of sodium hydroxide solution.	Formation of Yellow precipitate	Presence of Mercury
Test for Arsenic: 2m1 of the extract was taken in a test tube and treated with 2ml of sodium hydroxide solution.	Formation of brownish red precipitate	Presence of Arsenic

Results were noted and tabulated in Table No:10

Table No:5 Test for acidic radical

PROCEDURE	OBSERVATION	INFERENCE
Test for Sulphate: 2 ml of the extract was taken in clean, dry test tube and 5 % barium chloride solution was added to it.	Formation of white precipitate	Presence of Sulphate
Test for Chloride: The extract was taken in a test tube and then treated with Silver nitrate solution.	Formation of White precipitate	Presence of Chloride
Test for Phosphate: The extract was taken in a test tube and treated with ammonium molybdate and conc. HNO_3.	Formation of Yellow precipitate	Presence of Phosphate
Test for Carbonate : The substance was taken in a clean dry test tube and then treated with Conc. HCl.	Formation of Effervescence	Presence of Carbonate
Test for fluoride &oxalate: 2ml of extract was taken in a test tube and added with 2ml of dil.acetic acid, 2ml calcium chloride solution and then heated.	Formation of cloudy appearance	Presence of Fluoride & Oxalate
Test for Nitrate: 1gm of the *Rasaparpam* was heated with copper turnings andconcentrated H2SO4 and observed the test tube vertically down.	Characteristic changes	Presence of Nitrate

The bio-chemical analysis was done to identify the acid and basic radicals present in the RP

The results were tabulated in Table No: 11

4.2.3. Anti-microbial Load

Availability of microbial load [82]:

Enumeration of bacteria by plate count – agar plating technique

The plate count technique was one of the most routinely used procedures because of the enumeration of viable cells by this method.

Principle:

This method was based on the principle that when material containing bacteria are cultured, every viable bacterium develops into a visible colony on a nutrient agar medium. The number of colonies therefore was the same as the number of organisms contained in the sample.

Dilution:

A small measured volume is mixed with a large volume of sterile water or saline called the diluent or dilution blank. Dilution is usually made in multiples of ten. A single dilution was calculated as follows:

Volume of the sample Dilution = Total volume of the sample and the diluent

Requirements:

- Sample or Bacterial suspension
- 9 ml dilution blanks (7)
- Sterile petri dishes (12)
- Sterile 1 ml pipettes (7)
- Nutrient agar medium (200 ml) Colony counter.

Procedure:

- Label the dilution blanks as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}.
- Prepare the initial dilution by adding 1 ml of the sample into a 9 ml dilution blank labelled10^{-1} thus diluting the original sample 10 times.
- Mix the contents by rolling the tube back and forth between hands to obtain uniform distribution of organisms.

- From the first dilution transfer 1 ml of the suspension while in motion, to the dilution blank 10^{-2} with a sterile and fresh 1 ml pipette diluting the original specimen to 100 times.
- From the 10^{-2} suspension, transfer 1 ml of suspension to 10^{-3} dilution blank with a fresh sterile pipette, thus diluting the original sample to 1000 times.
- Repeat this procedure till the original sample has been diluted 10,000,000 times using every time a fresh sterile pipette.
- From the appropriate dilutions transfer 1ml of suspension while in motion, with the respective pipettes, to sterile petri dishes. Three petri dishes should be used for each dilution.
- Add approximately 15 ml of the nutrient medium, melted and cooled to 45◦c, to each petri dish containing the diluted sample. Mix the contents of each dish by rotating gently to distribute the cells throughout the medium.
- Allow the plates to solidity.
- Incubate these plates in an inverted position for 24-48 hours at 37◦C.

Observation

Observe all the plates for the appearance of bacterial colonies. Count the number of colonies in the plates.

Calculate the number of bacteria per ml of the original suspension as follows:

Number of colonies (average of 3 replates) Organisms per millimetre = Amount of plated × dilution

4.2.4. Sophisticated Instrumental Analysis

To know the test sample RP thoroughly – particle size, quantitative and qualitative values of chemical elements, molecular structure and their functional group, it underwent many analysis done by instruments.

FT-IR (Fourier Transform Infra-Red)

Definition:

FTIR offers quantitative and qualitative analysis for organic and inorganic samples. Fourier Transform Infrared Spectroscopy (FTIR) identifies chemical bonds in a molecule by producing an infrared absorption spectrum. The spectra produce a profile of the sample, a distinctive molecular fingerprint that can be used to screen and scan samples for many different components. FTIR is an effective analytical instrument for detecting functional groups[(83)].

Applications:

Quantitative Scans, Qualitative Scan Solids, Liquids, Gases Organic Samples, Inorganic Samples Unknown Identification Impurities Screening Formulation Pharmaceuticals.

Instrument details

It is the preferred method of infrared spectroscopy. FT-IR is an important and more advanced technique. It is used to identify the functional group, to determine the quality and consistency of the sample material and can determine the amount of compounds present in the sample. It is an excellent tool for quantitative analysis

In FT-IR infrared is passed from a source through a sample.

FTIR Instrument

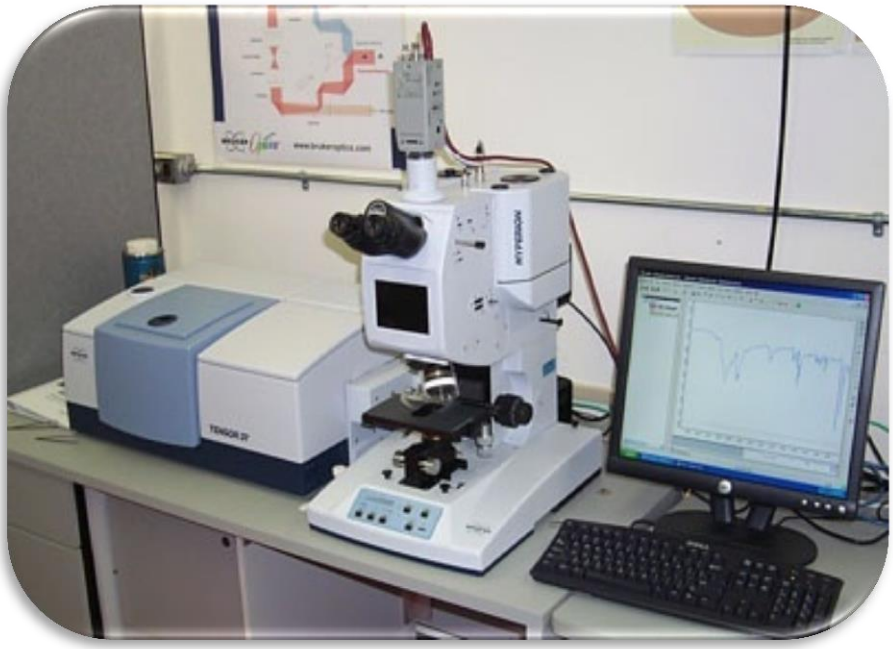


Fig No. 12. FTIR Instrument

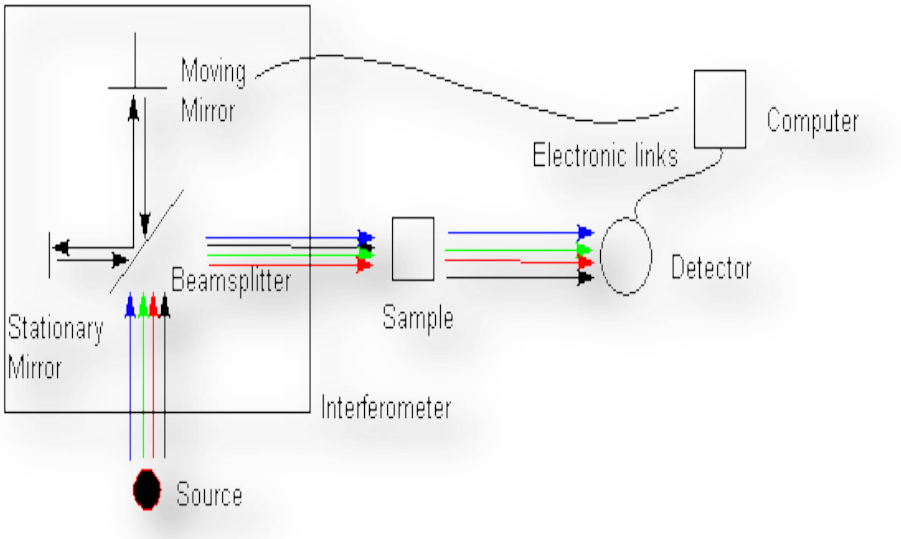


Fig No. 13. FTIR Mechanism

Model : Spectrum one: FT-IR Spectrometer Scan Range : MIR 450-4000 cm-1

Resolution : 1.0 cm-1 Sample required : 50 mg, solid or liquid.

This infrared is absorbed by the sample according to the chemical properties and some are transmitted. The spectrum that appears denotes the molecular absorption and transmission. It forms the molecular fingerprint of the sample. Like the finger print there is no two unique molecular structures producing the same infrared spectrum. It is recorded as the wavelength and the peaks seen in the spectrum indicates the amount of material present.

FT-IR is the most advanced and the major advantage is its

- Speed
- Sensitivity
- Mechanical
- Simplicity
- Internally Calibrated[(84)].

SEM (Scanning electron microscope)

Definition

Scanning Electron Microscopy (SEM), also known as SEM analysis or SEM microscopy, is used very effectively in microanalysis and failure analysis of solid inorganic materials. Scanning electron microscopy is performed at high magnifications, generates high-resolution images and precisely measures very small features and objects[(85)].

SEM analysis applications

The signals generated during SEM analysis produce a two-dimensional image and reveal information about the sample including:

External morphology (texture) Chemical composition (when used with EDS) Orientation of materials making up the sample

The EDS component of the system is applied in conjunction with SEM analysis to:

Determine elements in or on the surface of the sample for qualitative information Measure elemental composition for semi-quantitative results Identify foreign substances that are not organic in nature and coatings on metal. SEM Analysis with EDS – qualitative and semi-quantitative results Magnification – from 5x to 300,000x

Sample Size – up to 200 mm (7.87 in.) in diameter and 80 mm (3.14 in.) in height. Materials analysed – solid inorganic materials including metals and minerals.

Fig. No. 14. SEM Instrument

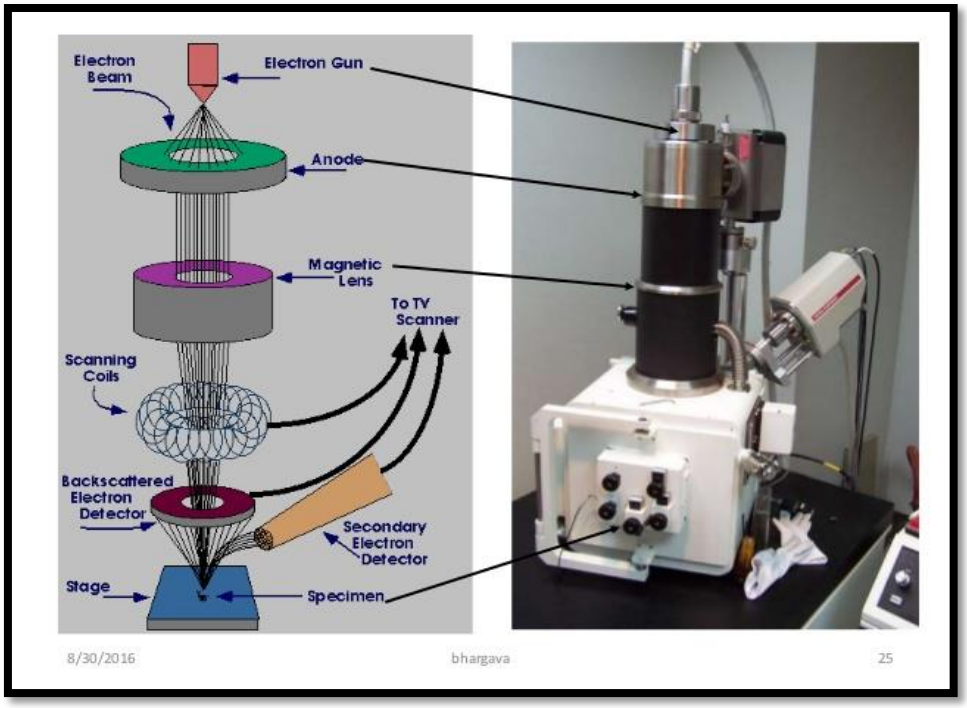


Fig. No.15. SEM Mechanism

The SEM analysis process

Scanning Electron Microscopy uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. In most SEM microscopy applications, data is collected over a selected area of the surface of the sample and a two-dimensional image is generated that displays spatial variations in properties including chemical characterization, texture and orientation of materials.

The SEM is also capable of performing analyses of selected point locations on the sample. This approach is especially useful in qualitatively or semi-quantitatively determining chemical compositions, crystalline structure and crystal orientations.

The EDS detector separates the characteristic X-rays of different elements into an energy spectrum and EDS system software is used to analyse the energy spectrum in order to determine the abundance of specific elements. A typical EDS spectrum is portrayed as a plot of X-ray counts vs. energy (in keV). Energy peaks correspond to the various elements in the sample.

Energy Dispersive X-ray Spectroscopy can be used to find the chemical composition of materials down to a spot size of a few microns and to create element composition maps over a much broader raster area. Together, these capabilities provide fundamental compositional information for a wide variety of materials, including polymers.

In scanning electron microscope high energy electron beam is focused through a probe towards the sample material. Variety of signals was produced on interaction with the surface of the sample. This results in the emission of electrons or photons and it is collected by an appropriate detector[(86)].

The types of signal produced by a scanning electron microscope include

Secondary electrons back scattered electrons characteristic x-rays, light specimen current Transmitted electrons.

FT-Raman/IR spectrophotometer [(87)]

This instrument is used to determine of molecular structure or to indentify materials by infrared spectroscopy and Raman spectroscopy. Generally: Fourier transformation Raman spectroscopy is suitable to investigate chemical composition

and structural properties of solid and liquid (polarization measurements) samples. The technique is non-destructive with no sample preparation requirement.

Specifically Dedicated BioRad FT-Raman spectrometer with near-IR excitation laser (1064 nm). The near-IR excitation is a requirement for biological samples allowing non-destructive spectra collection with reduced fluorescence.

The Raman scattering technique is a vibrational molecular spectroscopy which derives from an inelastic light scattering process. With Raman spectroscopy, a laser photon is scattered by a sample molecule and loses (or gains) energy during the process. The amount of energy lost is seen as a change in energy (wavelength) of the irradiating photon. This energy loss is characteristic for a particular bond in the molecule. Raman can best be thought of as producing a precise spectral fingerprint, unique to a molecule or indeed and individual molecular structure.

The *RASA PARPAM* obtained after sublimation process, shows 6 peaks respectively. The results are shown in Table.No.14.

Fig.No. 16. FT-Raman spectrometer

ICPOES (Inductively Coupled Plasma Optic Emission Spectrometry)

Fig No.17. ICP-OES Instrument

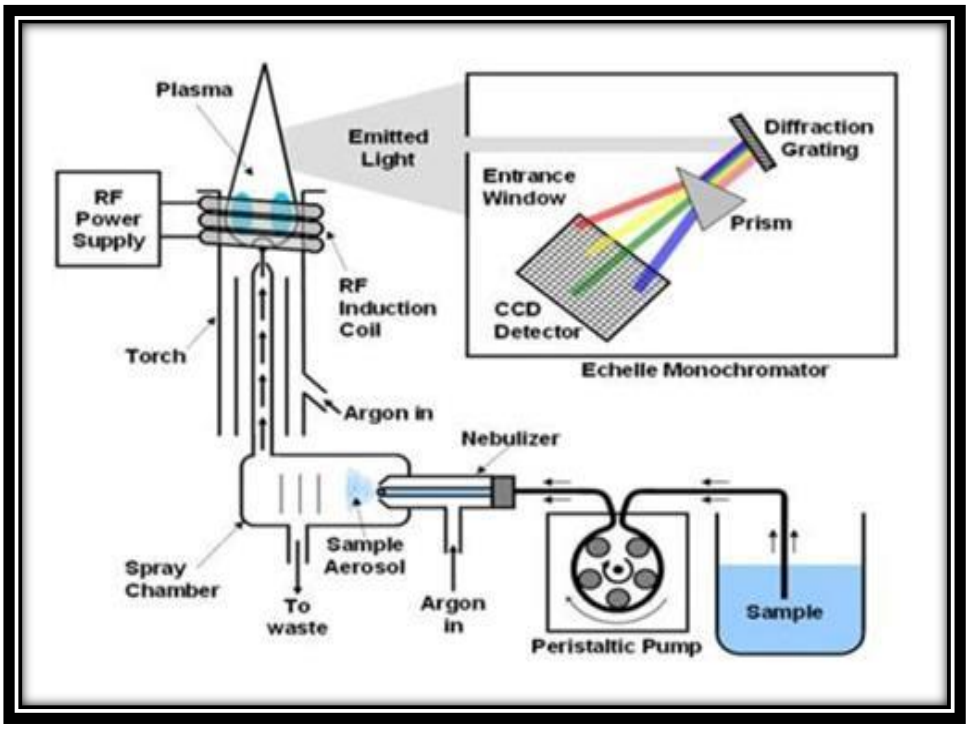


Fig No:18. ICP-OES Mechanism

Manufacturer: Perkin Elmer Model

Optima 5300 DV ICP-OES Inductively Coupled Plasma Spectrometer (ICP) Principle: An aqueous sample is converted to aerosols via a nebulizer. The aerosols are transported to the inductively coupled plasma which is a high temperature zone (8,000– 10,000ºC). The analysts are heated (excited) in different (atomic and/or ionic) states and produce characteristic optical emissions (lights). These releases are separated based on their respective wavelengths and their strengths are measured (spectrometry). The intensities are proportional to the concentrations of analyses in the aqueous sample.

The quantification is an external multipoint linear standardization by comparing the emission intensity of an unknown sample with that of a standard sample. Multi-element calibration standard solutions are prepared from single- and multi element primary standard solutions. With respect to other kinds of analysis where chemical speciation is relevant (such as the concentration of ferrous iron or Ferric Iron), only total essential concentration is analysed by ICP-OES[(88)].

Application

The analysis of major and minor elements in solution samples.

Objectives

- Determine elemental concentrations of different metals.
- Learn principles and operation of the ICP-OES instrument
- Develop and put on a method for the ICP-OES sample analysis
- Enhance the instrumental conditions for the analysis of different elements Probes the outer electronic structure of atoms.

Mechanism:

In plasma emission spectroscopy (OES), a sample solution is presented into the core of Inductively coupled argon plasma (ICP), which generates temperature of approximately 8000°C. At this temperature all elements become thermally excited and emit light at their characteristic wavelengths.

This light is collected by the spectrometer and passes through a diffraction grating that serves to resolve the light into a spectrum of its essential wavelengths. Within the

spectrometer, this deflected light is then collected by wavelength and amplified to yield an strength of measurement that can be converted to an elemental concentration by comparison with standardizationvalues. The Inductively coupled plasma optical emission spectrometric (ICP-OES) analysis was done in SAIF, IIT MADRAS, Chennai-36 using Perkin Elmer Optima 5300 DV.

Sample preparation:

Inductively Coupled Plasma Spectroscopy techniques are the so-called "wet" sampling methods whereby samples are introduced in liquid form for analysis. 100 mg RP was occupied in a clean, dry test tube. To this, 3 ml Nitric acid was added and mixed well and allowed for few minutes until the reactions were completed. And then, 25 ml of Refined water, was added to prepare digested solution.

The digested sample solution was shifted into plastic containers and labelled properly. It was completed in Bio-chemistry lab, Govt. Siddha Medical College, Chennai-106.

4.2.5. TOXICOLOGICAL STUDIES

Acute oral toxicity – OECD guidelines – 423

Acute toxicity study was carried out as per OECD guideline (Organization for Economic Co - operation and Development, Guideline-423[89].

IAEC No: IAEC/XLVIII/03/CLBMCP/2016,C.L.BaidMetha College of Pharmacy, Thoraipakkam, Chennai.

Introduction

- The acute toxic class method is a stepwise procedure with the use of 3 animals of a single sex per step.
- Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgement on the acute toxicity of the test substance.
- This procedure is reproducible, uses very few animals and is able to rank substances in a similar manner to the other acute toxicity testing methods.

- The acute toxic class method is based on biometric evaluations with fixed doses, adequately separated to enable a substance to be ranked for classification purposes and hazard assessment.
- In principle, the method is not intended to allow the calculation of a precise LD50, but does allow for the determination of defined exposure ranges where lethality is expected since death of a proportion of the animals is still the major endpoint of this test.
- The method allows for the determination of an LD50 value only when at least two doses result in mortality higher than 0% and lower than 100%.
- The use of a selection of pre-defined doses, regardless of test substance, with classification explicitly tied to number of animals observed in different states improves the opportunity for laboratory to laboratory reporting consistency and repeatability.

Principle of the Test

It is the principle of the test that based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.

- no further testing is needed
- dosing of three additional animals, with the same dose
- dosing of three additional animals at the next higher or the next lower dose level. The method will enable a judgment with respect to classifying the test substance to one of a series of toxicity classes.

Methodology

Selection of Animal Species

The preferred rodent species is the wistar albino rat, although other rodent species may be used. Healthy young adult animals are commonly used laboratory strains should be employed. Females should be nulliparous and non-pregnant. Each animal, at the commencement of its dosing, should be between 6 to 8 weeks old and the

weight (150-200gm) should fall in an interval within±20 % of the mean weight of any previously dosed animals.

Housing and Feeding Conditions

The temperature in the experimental animal room should be 22ºC ± 3ºC. Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. Animals may be group-caged by dose, but the number of animals per cage must not interfere with clear observations of each animal[(90)].

Preparation of Animals

The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 7 days prior to dosing to allow for acclimatization to the laboratory conditions

Test Animals and Test Conditions:

Sexually mature Female Wistar albino rats (150-200 gm) were obtained fromKings institute, Chennai.All the animals were kept under standard environmental condition (22±3°C). The animals had free access to water and standard pellet diet (Sai meera foods, Bangalore).

Preparation for Acute Toxicity Studies

Rats were deprived of food overnight (but not water 16-18 h) prior to administration of the, Rasa Parpam. The principles of laboratory animal care were followed and the Institutional Animal Ethical Committee approved the use of the animals and the study design.

Test Substance	**:** ***RASA PARPAM***
Animal Source	**:** Kings institute, Chennai.
Animals	**:** Wistar AlbinoRats (Female-3+3)
Age	**:** 6-8 weeks
Body Weight on Day 0	**:** 150-200gm.
Acclimatization	**:** Seven days prior to dosing.
Veterinary examination	**:** Prior and at the end of the acclimatization period.

Identification of animals **:** By cage number, animal number and individual marking by using Picric acid.

Numberofanimals **:** 3 Female/group,

Routeofadministration **:** Oral

Diet **:** Pellet feed supplied by Sai meera foods Pvt Ltd,Bangalore

Water **:** Aqua guard portable water in polypropylene bottles.

Housing & Environment **:** The animals were housed in Polypropylene cages provided with bedding of husk.

Housing temperature **:** Between 22ºC + 3ºC.

Relative humidity **:** Between 30% and 70%,

Air changes **:** 10 to 15 per hour and

Dark and light cycle **:** 12**:**12 hours.

Duration of the study **:** 14 Days

Administration of Doses

Rasa Parpamwas suspended in water and administered to the groups of wistar albino rats in a single oral dose by gavage using a feeding needle. The control group received an equal volume of the vehicle. Animals were fasted 12 hours prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. Three Female animals are used for each group. The dose level of 10µg/kg, 1 mg/kg and 2 mg/kgbody weight was administered stepwise.

After the substance has been administered, food was withheld for a further 3-4 hours. The principle of laboratory animal care was followed. Observations were made and recorded systematically and continuously as per the guideline after substance administration. The visual observations included skin changes, mobility, aggressively, sensitivity to sound and pain, as well as respiratory movements.Finally, the number of survivors was noted after 24 hrs and these animals were then monitered for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

Observations

Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the

first 4 hours, and daily thereafter, for a total of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead.

It should be determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed. All observations are systematically recorded with individual records being maintained for each animal.

Observations include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems and somatomotor activity and behavior pattern. Attention was directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. The principles and criteria summarized in the Humane Endpoints Guidance Document taken into consideration. Animals found in a moribund condition and animals showing severe pain or enduring signs of severe distress was humanly killed. When animals are killed for humanreasons or found dead, the time of death was recorded.

Behaviour:

The animals will be observed closely for behaviour in the first four hours which includes abnormal gait, aggressiveness, exophthalmos, ptosis, akinesia, catalepsy, convolusion, excitation, head twitches, lacrimation, loss of corneal reflex, loss of traction, piloerection reactivity of touch, salivation, scratching, sedation, chewing, head movements, sniffing, straub, tremor and writhes, diarrhea, leathery, sleep and coma.

Body Weight:

Individual weight of animals was determined before the test substance was administered and weights will be recorded at day 1, 7, and 14 of the study. Weight changes were calculated and recorded. At the end of the test, surviving animals were weighed and humanly killed.

Food and water Consumption

Food and water consumed per animal was calculated for control and the treated dose groups.

Mortality:

Animals were observed for mortality throughout the entire period.

Data and reporting

All the data were summarised in tabular form showing the animals used, number of animals displaying signs of toxicity, the number animals found dead during the test or killed for humane reasons, a description and the time course of toxic effects and reversibility, and necropsy findings.

Test substance and Vehicle In order to ensure the uniformity in drug distribution in the medium the suspension was made by mixing Rasaparpam with 2% CMC solution and it was found suitable for dose accuracy. Justification for choice of vehicle.

The vehicle selected as per the standard guideline is pharmacologically inert and easy to employ for new drug development and evaluation technique[91]. (Schlede E., Mischke U.,etal)

28-Days Repeated Oral Toxicity Study of

Rasa Parpam

Test Substance	: ***RASA PARPAM***
Animal Source	: Kings institute, Chennai.
Animals	: Wister Albino Rats (Male -24, and Female-24)
Age	**:** 6-8 weeks
Body Weight :150-200gm.	
Acclimatization	**:** Seven days prior to dose.
Veterinary examination	**:** Prior and at the end of the acclimatization period.
Identification of animals	**:** Bycage number, animal number and individual marking by using Picric acid
Diet	**:** Pellet feed supplied by Sai meera foods Pvt Ltd, Bangalore
Water	**:** Aqua guard portable water in polypropylene bottles.
Housing & Environment	**:** The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	**:** Between 22ºC $\pm$ 3ºC.
Relative humidity	**:** Between 30% and 70%,
Air changes	**:** 10 to 15 per hour

Dark and light cycle **:** 12**:**12 hours.

Duration of the study **: 28 Days.**

Table No.6. Animal groups

GROUPS	No.of Rats
Group I Vehicle control (water)	12 (6 male, 6 female)
Group II Rasa Parpam (10 µg/kg)	12 (6 male, 6 female)
Group III Rasa Parpam (100 mg/kg)	12 (6 male, 6 female)
Group IV Rasa Parpam 200mg/kg	12 (6 male, 6 female)

Methodology[(92)]

Randomization, Numbering and Grouping of Animals

48 Wistar Albino Rats (24M + 24F) were selected and divided into 4 groups. Each group consist of 12 animals (Male -6, and Female-6). First group treated as a control and other three group were treated with test drug (low, mid, high) for 28 days. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was marked with picric acid. The females were nulliparous and non-pregnant.

Justification for Dose Selection:

As per OECD guideline three dose levels were selected for the study. They are low dose (X), mid dose dose (100X), high dose (200X). X is calculated by multiplying the therapeutic dose (30mg) and the body surface area of the rat (0.018). i.e X dose is 8.784 µg/animal, (rounded to 10 µg), 100X dose is 1mg/animal,200X dose is 2mg/animal.

Preparation and Administration of Dose:

Rasa Parpam suspended in with water, It was administered to animals at the dose levels of X, 100X, 200X. The test substance suspensions were freshly prepared every two days once for 28 days. The control animals were administered vehicle only.

The drug was administered orally by using oral gavage oncedaily for 28 consecutive days.

Observations

Experimental animals were kept under observation throughout the course of study for the following:

Body Weight:

Weight of each rat was recorded on day 0, at weekly intervals throughout the course of study.

Food and water Consumption:

Food and water consumed per animal was calculated for control and the treated dose groups.

Clinical signs:

All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

Mortality:

All animals were observed twice daily for mortality during entire course of study.

Necropsy:

All the animals were sacrificed on day 29. Necropsy of all animals was carried out and the weights of the organs including liver, kidneys, spleen, brain, heart, and lungs were recorded.

The relative organ weight of each animal was then calculated as follow,

$$\text{Relative organ weight (g)} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of animal on sacrifice day (g)}} \times 100$$

Laboratory Investigations:

Following laboratory investigations were carried out on day 29 in animals fasted over-night. Blood samples were collected from orbital sinus using sodium heparin (200IU/ml) for Bio chemistry and potassium EDTA (1.5 mg/ml) for Hematology as anticoagulant. Blood samples were centrifuged at 3000 r.p.m. for 10 minutes.

Haematological Investigations:

Haematological parameters were determined using Haematologyanalyzer.

Biochemical Investigations:

Biochemical parameters were determined using auto-analyzer.

Histopathology:

Control and highest dose group animals will be initially subjected to histopathological investigations. If any abnormality found in the highest dose group than the low, then the mid dose group will also be examined. Organs will be collected from all animals and preserved in 10% buffered neutral formalin for 24 h and washed in running water for 24 h. The organ sliced 5 or 6µm sections and were dehydrated in an auto technicon and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained withHaematoxylin-eosin red.

Statistical analysis:

Findings such as body weight changes, water and food consumption,hematology and blood chemistry were subjected to One-way ANOVA followed by dunnett test using a computer software Graph pad version 7.

All data were summarized in tabular form, (Table.No.19 to Table No.23)

4.3. PHARMACOLOGICAL ACTIVITY

4.3.1. Pharmacological activity in-vitro Anticancer activity determination by MTT assay

HeLa (cervical cancer cells) was initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbeccos modified Eagles medium (Gibco, Invitrogen).

The HeLa cell line was cultured in 25 cm2 tissue culture flask with DMEM supplemented with 10% FBS L-glutamine, sodium bicarbonate and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphotericin B (2.5µg/ml). Cultured cell lines were kept at 37ºC in a humidified 5% CO2 incubator (NBS Eppendorf, Germany).

The viability of cells was evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT (4,5-dimethylthiazol-2-yl) assay method.

Cells seeding in 96 well plate: Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10% growth medium, 100µl cell suspension (5x104 cells/well) was seeded in 96 well tissue culture plate and incubated at 37ºC in a humidified 5% CO2 incubator.

Preparation of plant extracts and compound stock: 1 mg of sample/compound was added to 1ml of DMEM and dissolved completely by cyclomixer. After that the solution was filtered through 0.22 µm Millipore syringe filter to ensure the sterility.

Cytotoxicity Evaluation:

After 24 hours the growth medium was removed, freshly prepared each plant extracts in 5% DMEM were five times serially diluted by two-fold dilution (100µg, 50µg, 25µg, 12.5µg, 6.25µg in 100µl of 5% MEM) and each concentration of 100µl were added in triplicates to the respective wells and incubated at 37ºC in a humidified 5% CO2 incubator.

Cytotoxicity Assay by Direct Microscopic observation: Entire plate was observed at an interval of each 24 hours; up to 72 hours in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observation were recorded as images.

Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

Cytotoxicity Assay by MTT Method: Fifteen mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization.

After 24 hours of incubation period, the sample content in wells were removed and 30µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37ºC in a humidified 5% CO2 incubator for 4 hours.

After the incubation period, the supernatant was removed and 200µl of MTT Solubilisation Solution DMSO was added and the wells were mixed gently by pipetting up and down in order to solubilise the formazan crystals.

The absorbance values were measured by using microplate reader at a wavelength of 540 nm (Laura B. Talarico et al., 2004).

The percentage of growth inhibition was calculated using the formula:

$$\% \text{ of viability} = \frac{\text{Mean OD Samples}}{\text{Mean OD of control group}} \times 100$$

4.3.2. Anti- tumor activity

Anti-tumor activity Cell culture

The human cervical carcinoma a cell lines, HeLa and SiHa, used in the study was obtained from National Centre for Cell Science (NCCS), Pune, India. The cells were grown in DMEM containing 2m ML-glutamine supplemented with 10% fetal bovine serum and100U/ml of penicillin-streptomycin. The cellswere incubated in a humidified 5% CO2 incubator at37°C.

% of viability = x 100

Cell growth analysis

HeLa and SiHa cells were seeded at a density of 1 × 105 cells/ml in24-well plates in triplicates. Next day, the cells were dosed with different concentrations of *RP* (1.25,2.5 and 5.0ȝµg/ml) and grown for 6, 12 and 24 h. The cells were harvested and counted for viability using trypan blue dye exclusion method.

Colony formation assay

The cells were plated at a seeding density of 1 × 103 cells/ ml in 6-well plates. After 24 h, the cells were exposed to various concentrations of RP: 0, 10, 20, 40, and 80 ȝg/ ml. Plates were incubated at 37°C in a5% CO2 incubator for one week. This was followed by fixing the colonies with 4% paraformaldehyde and staining with0.5%crystal violet. The colonies were photographed with Sony DSC-S75 cyber-shotcamera[(90)].

Soft agar assay Control HeLa and SiHa cells (5×103cells/ml) as well as cells treated with different concentrations of RP (10-80 ȝg/ml) were mixed at 40°C with 0.35%agarose (DNA grade, GIBCOBRL, CA, USA) in culture medium and gelled at room temperature for 20min over a previously gelled layer of 0.5% agarose in culture medium in 6-wellplates.

After incubation for 10days, colonies were photographed directly using an Axiovert200M microscope (CarlZeiss, Germany) and counted.

Measurement of Apoptosis

The cells were plated at a seeding density of 5 × 105 cells/ well and treated with different concentrations of RP (0-80 ȝg/ml). After 24 h of treatment, the cells were harvested and washed with PBS twice. Cells were stained with Annexin VFITC following the manufacturer's instructions (Annexin V-FITC apoptosis kit #3, Invitro-gen) and analyzed for apoptosis by FACS using Cell Quest Software.

Statistical analysis

All experiments were performed in triplicates and repeated at least five times and the data were presented as mean ±SD. Statistical analysis was conducted with the Sigma Stat 3.5program (Systat Software, Inc.) using one- way ANOVA. The level used for comparisons was Į=0.05.

4.3.3. Anti-oxidant activity

DPPH assay (2, 2-diphenyl-1-picrylhydrazyl)

The radical scavenging activity of RP extracts was determined by using DPPH assay according to Change tal.(2001).

The decrease in the absorption of the DPPH solution after the addition of an anti-oxidant was measured at 517nm.Ascorbic acid (10mg/ml DMSO) was used as reference.

Principle

1,1Diphenyl 2- PicrylHydrazyl is a stable (in powder form) free radical with red color which turns yellow when scavenged.

The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an anti-oxidant (H- A) can be written as,

(DPPH)+ (H-A) DPPH-H +(A)

Anti-oxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases.

The degree of discoloration indicates the scavenging potential of the anti-oxidant compounds or extracts in terms of hydrogen donating ability.

Reagent preparation

0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100mlof ethanol.

Working procedure

Different volumes (1.25-20µg/µl) of RP extracts were made up to 40µl with DMSO and 2.96ml DPPH (0.1mM) solution was added.

Their action mixture was incubated in dark condition at room temperature for 20min.After 20min, the absorbance of the mixture was read at 517 nm. 3ml of DPPH was taken as control.

The % radical scavenging activity of the RP extracts was calculated using the follow

$$\% \text{ inhibition} = \frac{\text{Control- test}}{\text{Contol}} \times 100$$

Ingredients of Rasaparpam

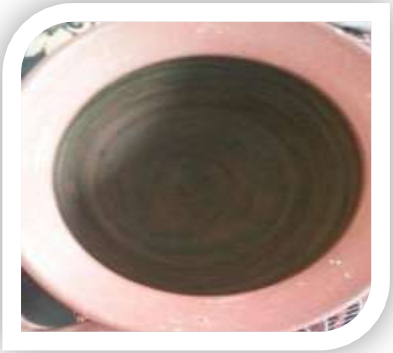
Fig.No.7. Purified Elemental Mercury

Fig.No.8. Sulfur

Fig.No.9. Urgineaindica

Fig.No. 7 – 9 Showing ingredients of *Rasaparpam*

Preparation of VaalaiRasam



(A) Herbal coating

(B) Ignition

(C) After ignition

(D) Collecting Vaalairasam

VaalaiRasam

Fig.No:10. A, B,C,D Showing preparation of Vaalairasam

Preparation of Rasaparpam

A. Grinding

B. Kuzhipudam

C. Incineration

D. Grinding

E. Pellet

F. Sealding

G. Ignition

RASAPARPAM

Fig.No:11. A – G Shows preparation of *Rasa Parpam*

5.RESULTS AND DISCUSSION

One of the Siddha Herbo mineral formulation of "*Rasaparpam*" had been exposed to scientific validates. Literary collection, Physicochemical and elemental analysis, toxicological studies and pharmacological studies are done to justify the anticancer activity of *RP* against cervical cancer.

From Review of literature

Discussion on *Gunapadam*review

- The poem for general properties of processed quicksilver directly indicates its anti-cancer nature.
- As per Siddha classical text, Sulfur by its herbo-mineral formulation indicates anti-cancer property.
- The plant *Urgineaindica*is used to treat cancer.

Discussion on modern drug review

- Mercury helps to destroy the cancer cells and reduces the tumor growth[(93)].
- Mercuric chloride has cyto toxiceffects[(94)].
- Sulfur contains anti-cancer property[(95)]
- *Urgineaindica* contains anti- oxidant property[(96)].

Discussion on pharmaceutical review

***Parpam*:**

- 100 years of shelf life denotes its long time efficacy.
- Being very fine particles in nature, increases the therapeutic effect.

Discussion of pharmacological review

The cell lines for anticancer activity were HeLa and SiHa. They are the genomes of HPV 16 and HPV 18 respectively. These HPV 16 and HPV 18 are responsible for 93% of Cancer cervix[(97)].

So, the analysis of pharmacological activity through HeLa and SiHa cell lines are the novel methods for validation which proves the effective anticancer activity of *RP*.

Discussion on materials and methods

- The selection of trial drug was taken from the book ***AathmaRakshamirthamEnnumVaithiyaSaaraSangiragam,*** Written by**KandhasamyMudhaliyar**, wasapproved by the Department of AYUSH as Per Classical Siddha literature.
- The ingredients were bought from the authenticated vender and they were identified and authenticated by the experts in Post Graduate Department Gunapadam, GSMC, Chennai. So the ingredients were perfect and original.
- The preparation of medicine was done at the well-equipped lab of the Post Graduate Department Gunapadam. So the principles of GMP were adhered during the process.
- The analytical parameters were conducted at registered and licensed laboratories only. Thus the result of *Rasaparpam* under various analytical procedures shows the accuracy of RP.
- The Siddha Herbo-mineral formulation *Rasaparpam* had been subjected to variousstudies for its scientific validation and safety assessment. Literary collections, physicochemical and Elemental analysis, Toxicological study, Pharmacological studies are done to prove its efficacy.

Table No.7.Results of Siddha Standardization

S.No	Parameters	Results of RP	Results of SP
1	Colour	White	White
2	Floating on water	Floats on water	Floats on water
3	Finger print test	Impinged in the furrow of fingers	Impinged in the furrow of the fingers
4	Luster	Lusterless	Lusterless
5	Taste	No specific taste	No specific taste

Interpretation

Colour:

Rasaparpam is white in colour. The absence of shining denotes that it is free from metals.

Floating on water:

*Rasaparpam*floats on water because of its less specific gravity. It denotes the lightness of the drug,So, it posesses the property of *Parpam*.

Finger print test:

Rasaparpam impinged on the lines of the finger. It is denotes the particles are fine and it is in micro size.

Luster :

No luster was observed in the *Rasaparpam*. It is due to the change of specific metallic character of raw material after incineration. It denotes that it is well manufactured.

Taste :

No specific taste in the *Rasaparpam*. It is due to the change of specific metallic character of raw material after incineration.

Table No.8.Physical characterization of *Rasaparpam*

S.No	Parameters	Result
1	Colour	White
2	State of the drug	Powder
3	Consistency	Fine powder
4	Solubility	
	Distilled water	Sparingly soluble
	Ethanol	Sparingly soluble
	Methanol	Sparingly soluble
	Propylene Glycol	Not soluble
	Petrolium ether	Not soluble
	Tolune	Not soluble
	Chloroform	Soluble
	Carbon tetra chloride	Soluble
	Xylene	Not soluble
5	Sense on touch	Fine

S.No	Parameters	Result
6	Sense on taste	Tasteless
7	Sense of smell	No significant smell is observed

Physicochemical Analysis

Table No.9.Results of Physicochemical Analysis

S.No	Parameters	Result
1	Loss on drying (at 105◦C)	1.0%
2	Total ash	99%
3	Water soluble ash	5.40%
4	Acid insoluble ash	3.65%
5	p^H	6.9

Interpretation

Solubility

- Solubility is the major factor that controls the bioavailability of a drugsubstance.
- It is useful to determine the form of drug and processing of its dosage form.
- The most frequent causes of low oral bioavailability are attributed to poor solubility and low permeability[(98)].
- *Rasaparpam*is soluble in major solvents and sparingly soluble in some solvents.This proves its efficiency of solubility in the stomach indirectly, increasing the bio availability.

P^H value

*Rasaparpam*shows acidic P^H.

- The P^H level plays a role in enzyme activity by maintaining the internal environment thus regulating the homeostasis.

- It is also an important factor for drug absorption [99]. Because of the acidic nature, the drug is more readily absorbed in an acidic medium like stomach which enhances the bioavailability of the drug.

Loss on drying

- Loss on drying (LOD) gives the total amount of volatile content and moisture (water) present in the drug.
- The stability of a drug and its shelf-life are dependent on moisture content. Moisture increase can adversely affect the active ingredient.
- Low moisture content- drug could get maximum stability and better shelf life.
- Since the drug has low loss on drying, the moisture content is less which is suitable for medicine preparation.

Ash values (Total Ash value)

Low total Ash value indicates the trial drug contains plant organic derivatives. It is not subjected to calcination process.

Acid insoluble ash

Lower the acid insoluble value better will be the drug quality[100]. The drug ensures a low value of acid insoluble ash indicating that the preparation did not contain any sand, dust and stones.

Water soluble ash

Decreased water soluble ash value (5.40 %) indicates easy facilitation of diffusion and osmosis mechanisms.

Biochemical Analysis

Table No.10. Results of Basic radicals

S.No	Parameters	Result
1	Test for Potassium	Negative
2	Test for Calcium	Positive
3	Test for Magnesium	Positive
4	Test for Ammonium	Negative

S.No	Parameters	Result
5	Test for Sodium	Negative
6	Test for Iron (ferrous)	Negative
7	Test for Zinc	Positive
8	Test for Aluminium	Positive
9	Test for Lead	Negative
10	Test for Copper	Negative
11	Test for Mercury	Positive
12	Test for Arsenic	Negative

The biochemical analysis for basic radicals of *RP* shows the presence of Calcium, Magnesium, Zinc, Aluminium and Mercury.

Table No.11.Results of Acid Radicals

S.No	Parameters	Result
1	Test for Sulphate	Positive
2	Test for Chloride	positive
3	Test for Phospate	Negative
4	Test for Carbonate	Negative
5	Test for Fluoride & Oxalate	Negative
6	Test for Nitrate	Negative

The bio chemical analysis for acid radical of *RP* shows the presence of Sulphate and Chloride.

Interpretation

The presence of these radicals helps*RPf*or its therapeutic effect.

- Zinc is needed for immune function, wound healing and blood clotting.
- Mercury helps to destroy the cancercells and reduces the tumor growth
- Mercuric chloride has cyto toxic effects
- Sulphate contains anti-cancer property.

Anti- microbial load

Availability of bacterial and fungal load in Rasaparpam

Table No.12. Bacterial and Fungal dilution

Micobes	Dilution	Result
Bacteria	10^{-4}	7
Bacteria	10^{-6}	6
Fungi	10^{-3}	Nil
Fungi	10^{-2}	Nil

Interpretation

- This is one of the Siddhaherbo mineral formulations which are prepared by using plant materials that are prone to contamination. The contamination of herbal drugs by micro organism not only cause bio deterioration but also reduces the efficacy of drugs.
- The toxic effect produced by microbes makes the herbal drugs to give no response for human consumption because the contaminated drug may develop unwanted disease instead of disease being cured.

FTIR(Fourier transform infrared spectroscopy)

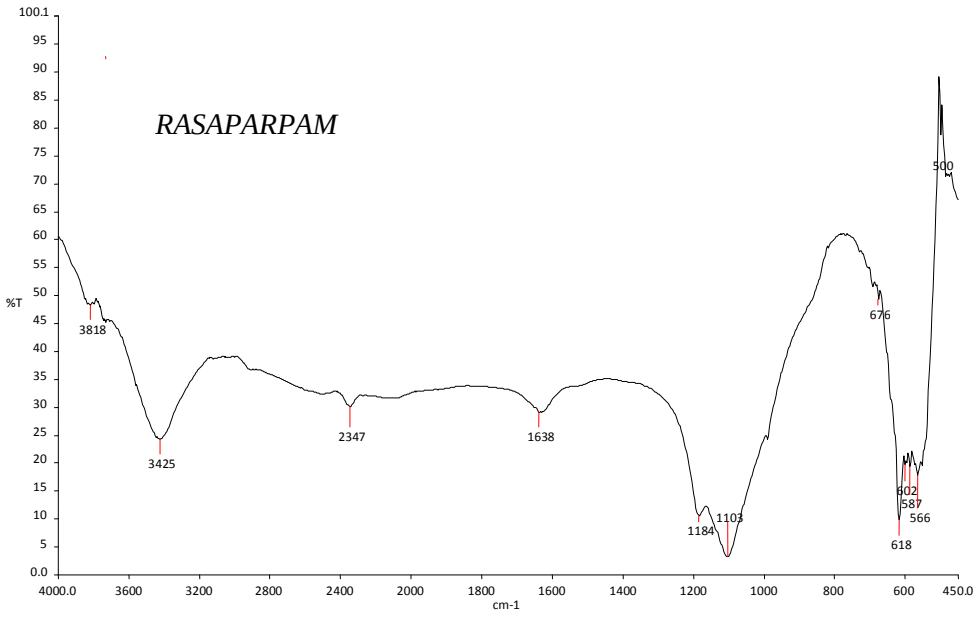


Fig.No.19.FTIR – waves of RP.

Table.13.FTIR- results of RP

Frequency, cm^{-1}	**Bond**	**Functional Group**
3425	O-H Stretch,H-bonded	Alcohol, Phenols
1638	N-H bend	1° amines
1184	C-H wag (-CH_2X)	Alkyl halides
1103	C-O Stretch	Carboxylic acids, esters,ether
676	C-H "oop"	Aromatics
618	-C=C-H:C-H bend	Alkynes
602	C-Br stretch	Alkyl halides
587	C-Cl stretch	Alkyl halides

Interpretation

- The wave numbers from 4000cm-1 to 1500cm-1 gives details for identification of functional group.
- The wave number from 1500cm -1 to 400 cm-1 provides particulars about molecular fingerprint.
- The above result showed the presence of functional group like Phenol, Alcohols, Alkynes, Amines, Carboxylic acids, Esters, Ether and Alkyl halides in*Rasaparpam*.
- They may be responsible for the presence of anticancer action of *RP* in cervical cancer.

Alcohols

- OH group of *RP*has higherr potential towards inhibitory activity against microorganism.

Phenols

- Phenols of *RP* possess highly Anti-Oxidant property which enchances its effect against the disease.
- Phenolic acid components take part in important roles in the control of cancer and other diseases.
- Currently great awareness is on the effect of phenols due to their anti-oxidative and possibeanti cancer activities.
- Free radicals react easily with phenols to abstract the hydrogen atom from the OH group.
- Phenolic acids and flavanoids also work as reducing agents, free radical scavengers and quenchers of single oxygen formation (Ali Ghasemzadehet al 2011)
- Phenols and flavanoidsposses diverse biological activities, for example, anti ulcer, anti-inflammatory, anti-oxidant, cytotoxic and anti-tumor, anti-spasmodic and anti-depressant activities.
- Phenols are the most important groups of secondary metabolites and bioactive compounds. Hydroquinone is one of the phenolic group inhibits the free radical reactions. (CHO7Alchohol HTI). It is also an antioxidant substance capable of scavenging free superoxide radicals, anti-aging and reducing the risk of cancer.

Carboxylic Acid

- Benzene-poly-carboxylic Acid Complex (BP-CI) is a novel anticancer complex against human cancer cells.
- Docosahexaenoic acid (DHA) is an omega-3 fatty acid. Its structure is a carboxylic acid (-oic acid) with a 22- carbon chain (docosa-is Greek for 22) and six (hexa-) cis double bounds [].
- DHA was revealed to increase the efficacy of chemotherapy in prostate cancer cells and a chemo protective effect in a mouse model was reported.
- It may also be used as a non- toxic adjuvant to increase the efficacy of chemotherapy.
- In mice, DHA was found to reduce growth of human colon carcinoma cells The cytotoxic effect of DHA was caused by decrease in cell growth regulators.

Ether:

- Certain ether lipids such as 1-0-octadecyl-2-0 methyl-rec-glycero-3-phosphocholine represent a new class of anti -neoplastic agents. These ether lipids have been shown to be cytotoxic for a wide variety of tumors.

Alkyl halides :

- High propotionof low molecular weight alkyl halides may be weakly carcinogenic and provide evidence supporting and eletrophillic hypothesis of carcinogenesis[(101)].

SEM (Scanning electron microscope)

The following image is done by 80000X via 500nm aperture shows maximum depth focused.

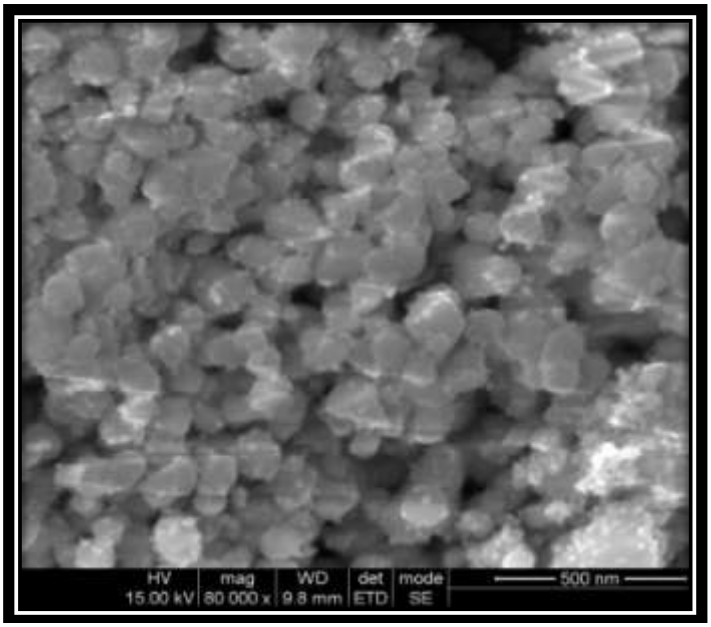


Fig.No.20. Showing nano particles in SEM image of 500nm

The particle size ranging from 80nm-120nm

Interpretation

- Nanoparticles, according to the American Society for Testing and Materials (ASTM) standard definition, are particles with lengths that range from 1 to 100 nm in two or three dimensions.

Advantages of nano particles

- Enhancing solubility of hydrophobic drugs,
- Prolonging circulation time,
- Preventing undesirable side effects,
- Minimizing nonspecific uptake,
- Specific cancer targeting[102].
- Improvingintra cellular penetration,
- The test drug *Rasaparpam* contains nanoparticle
- Nano particles present in the drug results in a better bioavailability and facilitates absorption.
- Nanotechnology a promising way from cancer management towards cancer elimination.
- The particles of nano size show that the drug may easily enter the cells at the molecular level to treat the disease rapidly and increase the therapeutic effect.

ICP-OES Results and Interpretation

Table No.14.ICP-OES Resultsof *RP*

S.No	Elements	Detected levels
1	Aluminium	BDL
2	Arsenic	BDL
3	Cadmium	BDL
4	Copper	BDL
5	Mercury	13.879mg/L
6	Potassium	03.821 mg/L
7	Sodium	04.300 mg/L
8	Nickel	BDL
9	Lead	BDL
10	Sulfur	70.304 mg/L

Interpretation

From the above results, the heavy metals Arsenic, Cadmium and Lead were found below detection level. Mercury, Sodium and Potassium are observed within the permissible limits.

Sulfur

Sulphur is commonly used in Asia as an herbal medicine to treat inflammation and cancer.

- Organic Sulfur has been studied on oral and other cancers and has been found to have remarkable benefit in anti-cancer therapy.
- Hence the safety of the drug *Rasaparpam* is ensured.

Raman Spectroscope – results of RP

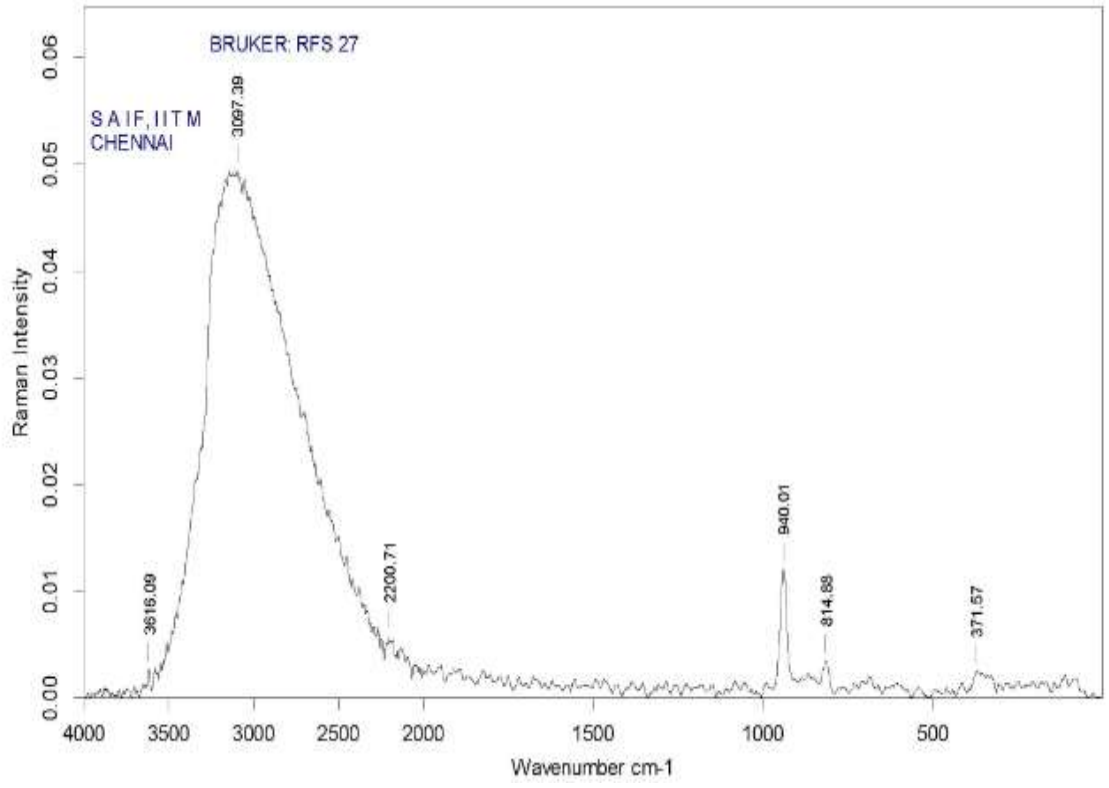


Fig.No:21. Raman spectrometer peaks of RP.

Table No.15. Raman Spectroscope – results of RP

Frequency, cm^{-1}	Functional group	Raman Bond
3616.09	O-H	Weak
3097.39	=C-H	Strong
2200.71	C=C	Strong
940.01	C-O-C	Medium
814.89	C-O-C	Medium
371.57	(CC)Aliphatic chains	Strong

Interpretation

- The drug obtained after sublimation process as per standard Siddha- SOP shows carbon linkages with Hydrogen, Carbon, Oxygen and aliphatic chains.
- These bond natures might be Ionic, Covalant, coordinate covalent (C-O-C bond linkage, C=C bond linkage)
- The sublimation process convert the heterogeneous material into homogeneous substance and creating new complex compounds of various bonds.
- Electronegativity is a measure of the tendency of an atom to attract a bonding pair of electrons.
- The bonding pair of electrons of one atom is pulled towards the other end of the atom bond.
- The larger the difference in electro negativity between the two atoms involved in the bond, makes it more ionic.
- This electronegativity/nucleophillic property of the *Rasa parpam* makes free radicals to get attracted by this electron clouds and some of the new bonding make hold the metabolites from damaging the cells and detoxify them and also these characters favours the phenomenon of electron transport chain and maintain the cellular metabolism properlyand ensures the Anti-Oxidant property.
- Essential propertiesof anti-oxidants:Oxidative stress induced cell damage through damage to proteins, lipids and DNA. It may also alter signaling

pathways redox sensitive to changes involved in the response of apoptosis. The antioxidants are currently the subject of many studies because, in addition to some interest in the preservation of comestibles, they could be useful in the prophylaxis and treatment of diseases in which oxidative stress is implicated.

- From the present study, it was concluded that the *Rasaparpam* extract has good anti-oxidant activity at higher concentrations.
- So, the*Rasaparpam* due to its anti-oxidant property could eliminate cancer cells.

ACUTE ORAL TOXICITY

Dose finding experiment and its behavioral signs of Toxicity for *Rasaparpam.*

Observation done

Table No.16. Observational study

S.No	**Group**	**Day**
1	Body weight	Slightly decreased
2	Assesments of posture	Normal
3	Signs of convulsion Limb paralysis	No signs of convulsion and paralysis
4	Body tone	Normal
5	Lacrimation	Slightly increased
6	Salivation	Normal
7	Change in skin colour	Normal
8	Piloerection	Abnormal
9	Defection	Normal
10	Sensitivity response	Normal
11	Locomotion	Normal
12	Muscle gripe	Normal
13	Rearing	Normal
14	Urinatiom	Normal

Table No.17. Observational study Results

Dose mg/kg	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
RP 200 mg/kg	+	-	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-

1.Alertness 2. Aggressiveness 3. Pile erection 4. Grooming 5.Gripping 6. Touch Response 7. Decreased Motor Activity 8. Tremors 9.Convulsions 10. Muscle Spasm 11.Catatonia 12.Muscle relaxant 13. Hypnosis 14.Analgesia 15.Lacrimation 16.Exophthalmos 17.Diarrhea 18.Writhing 19.Respiration 20.Mortality.

(+ Present, - Absent)

Table No.18.Body weight observation

DOSE	**DAYS**		
	1	7	14
CONTROL	180.6±1.44	181.4 ± 4.32	183.2 ± 7.63
HIGH DOSE	190.5± 7.75	188.7 ± 1.67^{ns}	184.4 ± 2.67^{ns}

Acute toxicity Discussion

- In the acute toxicity study, the rats were treated with different concentration of *Rasa Parpam*from the range of 5mg/kg to 200mg/kg.
- This dose level did not produce signs of toxicity, behavioral changes and mortality in the test groups as compared to the controls when observed during 14 days of the acute toxicity experimental period.
- These results showed that a single oral dose of the extract showed no mortality of these rats even under higher dosage levels indicating the high margin of safety of this extract.
- In acute toxicity test *Rasaparpam*was found to be non toxic at the dose level of 200mg/ kg body weight.

28 days Repeated Oral Toxicity of Rasaparpam

Table No.19.Body weight of wistar albino rats group exposed to *RP*

DOSE	DAYS				
	1	7	14	21	28
CONTROL	180.6±3.62	181.4 ± 4.14	183.7 ± 9.61	184.6 ± 3.03	185.7 ± 1.31
LOW DOSE	183.2 ± 1.14	181.4 ± 2.12	179.6±2.36	177.2 ± 4.78*	174.12± 2.39**
MID DOSE	186.6± 1.64	181.3 ± 2.74	179.4 ± 8.32	174.1 ± 3.16*	172.7 ± 3.82**
HIGH DOSE	184.4± 6.74	179.7 ± 3.64	176.4 ± 1.51	170.1 ± 4.66*	164.4± 3.76**

*NS- Not Significant, **(p > 0.01),*(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table No.20. Haematological parameters of Wistar albino rats group exposed to *RP*

Category	Control	Low dose	Mid dose	High dose
Haemoglobin(g/dl)	13.8±0.88	13.90±1.16	11.14±0.66	9.28±1.16*
Total WBC ($\times 10^3$ l)	11.91±0.59	11.85±1.23	10.08±1.21	8.110±2.27*
Neutrophils(%)	33.65±0.06	33.3±1.24	32.11±2.16	30.20±1.10
lymphocyte (%)	70.24±1.48	70.02±1.12	69.20±1.16	60±1.26*
Monocyte (%)	0.86±0.07	0.85±0.19	0.72±0.13	0.71±0.60
Eosinophil(%)	0.54±0.09	0.54±0.12	0.62±0.16	0.72±0.04
Platelets cells10^3/µl	687.17±8.76	678.71±9.16	623.18±2.20	627.16±3.74
Total RBC 10^6/µl	7.99±0.12	7.79±1.57	7.62±0.19	6.05±0.12*
PCV%	37.79±0.6	37.35±1.23	32.98±1.18	25.82±2.14*
MCHC g/dL	33.6±2.23	33.29±1.19	30.18±1.12	34.03±1.14
MCV fL(µm^3)	49.07±3.64	47.28±8.12	45.20±1.24	4.22±1.94

*N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table No.21. Biochemical Parameters of Wistar albino rats group exposed to *Rasa Parpam*

BIOCHEMICAL PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
GLUCOSE (R) (mg/dl)	74.45±13.4	78.16±1.24	92.26±1.22	110.12±9.60
T.CHOLOSTEROL(mg/dl)	115.26±1.83	118.45±1.13	132.42±1.78	156.22±1.93
TRIGLY(mg/dl)	46.35±1.48	48.22±1.28	49.58±1.80	59.66±1.13*
LDL	73.8±2.43	75.24±3.14	82.14±1.24	96.64±4.12*
VLDL	15.2±2.44	15.82±1.14	18.44±2.14	19.24±4.16
HDL	26.66±6.88	26.16±1.24	24.68±2.16	20.78±1.12*
Albumin(g/dL)	3.3±0.17	3.23±0.22	2.48±2.02	2.14±3.16*

*NS- Not Significant,**(p > 0.01), * (p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table No.22.Renal function test of Wistar albino rats group exposed to *RP*

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
UREA (mg/dl)	13.35±0.99	14.81±1.26	16.26±1.18	21.28±3.12*
CREATININE(mg/dl)	0.58±0.08	0.48±0.06	0.72±0.14	0.94±0.12*
BUN(mg/dL)	15.12±0.10	15.12±0.28	16.28±0.14	16.90±1.22
URIC ACID(mg/dl)	5.37±0.35	5.11±0.43	6.72±2.15	7.28±0.14*

*NS- Not Significant, **(p > 0.01), * (p >0.05) , n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table No.23.Liver Function Test ofWistar albino rats group exposed to *Rasa Parpam*

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
T BILIRUBIN(mg/dl).	0.50±0.07	0.58±0.16	0.62±0.18	0.76±0.15*
SGOT/AST(U/L)	114.95±1.39	118.15±2.11	131.21±1.23	145.55±1.23*
SGPT/ALT(U/L)	71.23±1.28	76.91±1.59	82.34±2.18*	86.32±1.28*
ALP(U/L)	146.25±8.77	144.2±6.27	149.16±4.17*	153.3±4.25*
T.PROTEIN(g/dL)	6.32±0.38	6.12±1.34	5.76±0.23*	5.10±1.26*

*NS- Not Significant, **(p > 0.01), * (p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Interpretation

- The dose selected for the sub acute toxicity study was 20mg, 40mg/kg of *Rasaparpam.*
- All the animals were free of intoxicating signs throughout the dosing period of 28 days.
- No physical changes were observed throughout the dosing period.
- Mild toxic but no mortality was observed during the whole experiment. No abnormal deviations were observed.
- No significant changes were observed in the values of different parameters studied when compared with controls and values obtained were within normal biological and laboratory limits.
- The weights of organs recorded that shows mild differences in the treatment when compared to control group. This indicates that *NamachivayaChendooram*induce mild changes in liver and kidney but not toxic to rest ofthe organs.
- There was slight changes were observed in hemoglobin (Hb), red blood cell (RBC). No significant changes in white blood cell (WBC), packed cell volume

(PCV), Erythrocyte sedimentation rate (ESR) in all the treated groups as compared to respective control groups.

Histopathology Examination

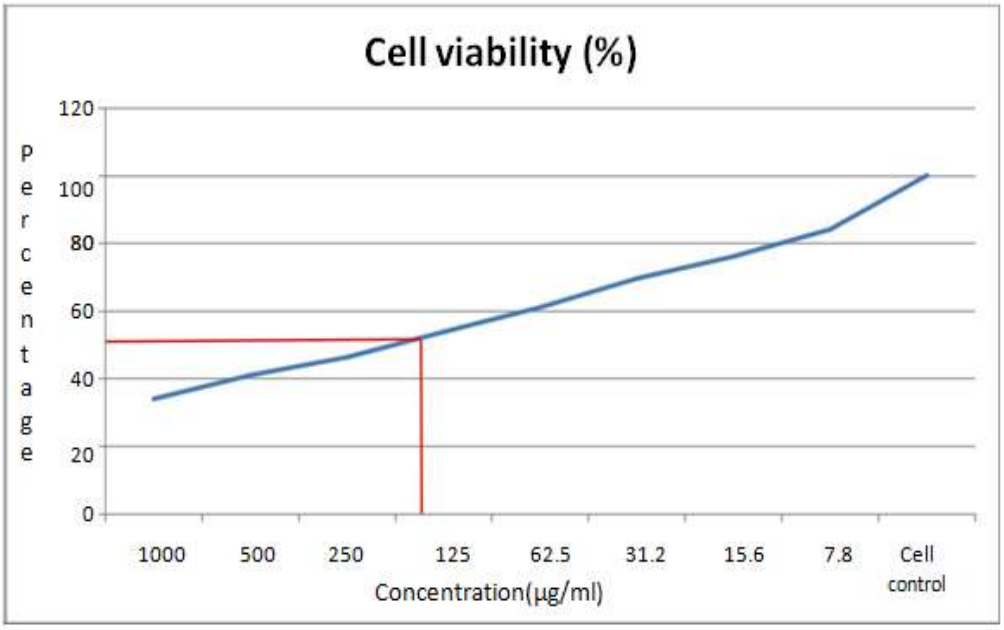
- Histopathology studies were carried out on liver, kidney and spleen were recorded. Blood samples for haemotological and bio chemical analysis were taken from common carotid artery.
- All tissues were preserved in 10% neutral buffered fomaldehyde solution for histopathological examination. The internal organs and tissues were observed for gross lesions.

PHARMACOLOGICAL STUDY

Table No.24. Anticancer effect of *Rasaparpamon HeLa* cell line

S.No.	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	1000	Neat	0.495	34.13
2	500	1:1	0.595	41.03
3	250	1:2	0.672	46.34
4	125	1:4	0.781	**53.86**
5	62.5	1:8	0.887	61.17
6	31.2	1:16	1.008	69.51
7	15.6	1:32	1.105	76.20
8	7.8	1:64	1.219	84.06
9	Cell control	-	1.450	100

- The percentage of growth inhibition was found to be increasing with increasing concentrations of test drug . The IC_{50} of test sample in HeLa cell line was found to be 125 µg/ml. This confirms that the Siddha formulation *Rasaparpam*has promising anti-cancerous effect.



Graph 1

Graph-1 *shows the drug dose and % of Inhibition of HeLa cells after theRasa parpam extract treatment. It can be observed by the result of MTT assay that the IC dose of Rasa parpam is 125µg/ml. As the dose increases the HeLa cell viability decreases. It was found that the % growth inhibition increasing with increasing concentration of Rasaparpam steadily up to 7.8 µg/ml on HeLa cell line (Table No: (24) and Graph(1) and that IC value on HeLa cell line was 50 and R value was 1.450.*

Interpretation

Cytotoxic effect by MTT assay:

- MTT ia a yellow water soluble tetrazolium salt. Succinate dehydrogenase , a mitochondrial enzyme in living cells, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells.
- This assay is based on the metabolic reduction of 3- (4,5-dimethylthiazol-2-yl) -2,5-difeniltetrazol (MTT) by mitochondrial enzyme succinate dehydrogenase in a coloured compound blue (formazan), allowing to determine the functionality of the mitochondrial treated cells. This method has been widely used to measure survival and cell proliferation.

- The amount of living cells is proportional to the amount of formazan produced. Cell lines derived from NCCS, Pune were free from any kind of bacterial and fungal contamination.
- To determine the cytotoxic effect of novel Siddha formulation *Rasa parpam* against HeLa cells. The experiment was screened at different concentrations to determine the IC_{50} using MTT assay. A chart was plotted using the % cell vibility in Y-axis and concentration of the test sample in X-axis.
- The percentage of growth inhibition was found to be increasing with increasing concentrations of test drug . The IC_{50} of test sample in HeLa cell line was found to be 125 µg/ml. This confirms that the Siddha formulation *Rasaparpam* has promising anti-cancerous effect.
- *RASA PARPAM* at different doses (7.8-1000 µg/ml of 5% MEM)was administered for 24 hrs. It was found that the number of cells decreases as the dose increases and at approximately 125 µg/ml dose of extract, 50% of the cells (HeLa cells) were less as compared to normal control as shown in Fig.No:23.
- The percentage of cells viability was determined by calculating the O.D of treated against thecontrol.
- Reading optical density (OD) is performed in a spectrophotometer at a wavelength of 570 nm.Comparison values are made on a basis of 50% inhibition of growth (IC_{50}) in treated cells with specific agents.Results are tabulated in Table (24) and graphically represented in Graph(1).

Analysis of Membrane Morphological Characteristics by Haematoxylin /Eosin (H/E) Staining

- Morphological changes such as changes to the cell membrane, loss of membrane asymmetry and cell shrinkage, are the early stage of apoptosis was analyzed by H/E staining.
- The IC dose (125µg/ml) treated cancer cells show features of apoptosis whereas treated with same amount of dose, to normal treated cells appeared without any significant changes.
- Since the discovery of the Cisplatin anti-tumor activity, great efforts have focused on the rational design of metal-based anticancer agents that can be potentially used in cancer chemotherapy.

- Over the last four decades, a large number of metal complexes have been extensively investigated and evaluated *in- vitro* and *in- vivo.*[103]
- The key focuses of these studies lie in finding novel metal complexes which could potentially overcome the hurdles of current clinical drugs including toxicity, resistance and other pharmacological deficiencies.
- Metals and metalic compounds have been used in medicine for several thousands of years. The medicinal uses and applications of metals and metal complexes are of increasing clinical and commercial importance.
- Monographs and major reviews, as well as dedicated volumes, testify to the growing importance of the discipline[104][105].
- Relevant reviews are to be found throughout annual series, for example Metal Ions in Biological Systems [106].
- The field of inorganic chemistry in medicine may usefully be divided into two main categories: firstly, ligands as drugs which target metal ions in some form, whether free or protein-bound and secondly, metal-based drugs and imaging agents where the central metal ion is usually the key feature of the mechanism of action [107].
- Arsenic has been used therapeutically for more than 2,000 years and was used in the 1930s for treatment of chronic myeloid leukemia until supplemented by newer chemotherapies [108].
- Side effects are cardiotoxicity, skin rashes, and hyperglycemia[109].
- Oncologists and scientists engaged in the research of cancer treatments should conduct a comprehensive study on the efficacy of Mercury which is being used as an anti-cancer drug in the age old Siddha system.
- Three years of research has shown that metal (Mercury, Arsenic and Copper) based Siddha drug is a safe alternative for Cisplatin therapy or Arsenic trioxide in selected cases of cancer treatments wherein the patients cannot bear the adverse effects.
- He found that mice treated with Siddha drugs showed better health than what did in Cisplatin therapy in terms of appetite, haemoglobin, red blood cells and white blood cells[110].

Anti-tumor activity

Rasaparpam Induced HeLa and SiHa cell Apoptosis

Cell Apoptosis

Cell apoptosis was analyzed by flow cytometry based on the Annexin V-FITC/ propidium iodide apoptosis kit. Briefly, the HeLa cells and SiHa cells were treated by RP (1.25,2.5 and 5.0µg/ml) then collected and washed twice by ice-cold PBS. After that, the cells were stained with Annexin V-FITC / propidium iodide. Apoptosis was evaluated by a flow cytometry assay.

Interpretation

- In this study, Annexin V/PI binding was used to evaluate the apoptosis of the cancer cells induced by RP. As shown in Figure , the percentage of apoptotic cells were significantly increased from 3.92% ± 2.09% to 22.86% ± 3.01%, 23.68% ± 4.5% and 66.03% ± 4.51% after the HeLa cells were treated with RP (1.25,2.5 and 5.0µg/ml) for 12 h.
- In addition, the percentages of apoptotic cells were significantly increased from 10.33% ± 6.39% to 38.92% ± 12.39%, 47.13% ± 13.61% and 56.99% ± 14.24% after the SiHa cells treated with RP (1.25,2.5 and 5.0µg/ml) for 24 h.

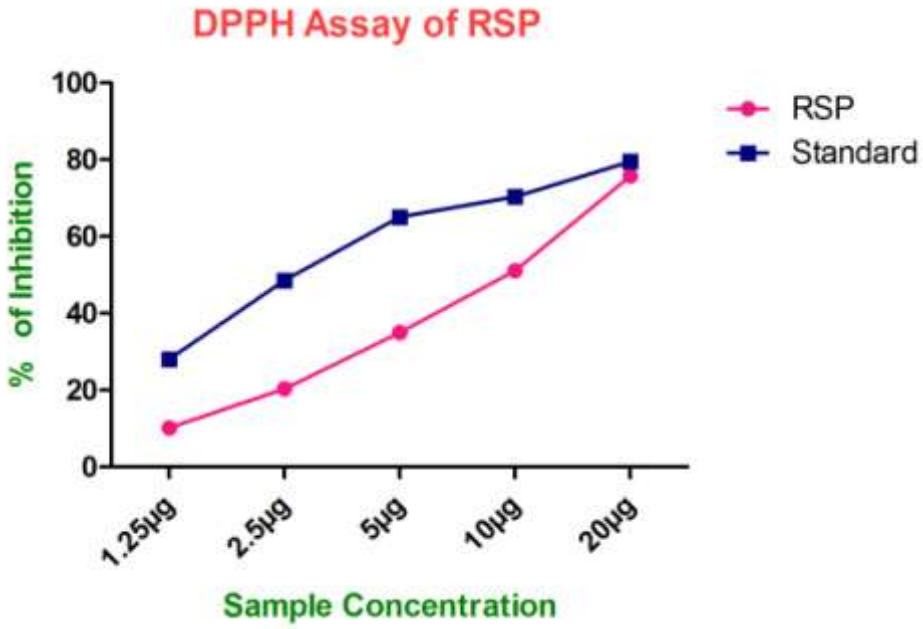
All these above data indicated that RP altered the growth kinetics of HeLa and SiHa cells in a significant manner that could be a positive indicator for testing its anti-tumor activity in cervical cancer cell.

Anti-Oxidant activity Activity

Table No.25. DPPH Assay of *Rasaparpam*

Concentration (µg/ml)	**Absorbance**		**Percentage of Inhibition**	
Rasaparpam	**Drug**	**Standard**	**Drug**	**Standard**
Control	0.5432	0.327	-	-
1.25	0.4876	0.235	10.23	28.14
2.50	0.4327	0.168	20.34	48.63
5.00	0.3527	0.114	35.07	65.14**
10	0.2653	0.097	51.16*	70.34
20	0.1317	0.067	75.75	79.52

(µg/ml) microgram per mililiter.Drug:RP (1.25µg/ml-20µg/ml) Standard: Ascarbic acid(10mg/mlDMSO)



Graph-2

DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of *Rasaparpam* extract. The antioxidant molecules can quench DPPH free radicals by providing hydrogen atom or by electron

donation and a colorless stable molecule 1, 1 diphenyl-2-picryl hydrazyl is formed and as a result to which the absorbance at 517 nm of the solution is decreased.

In the present study the *Rasaparpam* extract was analyzed was able to decolorize DPPH and the free radical scavenging activit y was expressed as the percentage decrease in absorbance.

(10mg/mlDMSO) have a concentration-dependent anti-radical activity which was tabulated in Table No.25

A maximum of 75.75% and 79.52 % anti-radical effects are exercised by *Rasaparpam* and standard drug ascorbic acid at concentrations of 20 µg / ml respectively. Minimum percentage of inhibition 10.23% and 28.14% anti-radical effects are manifested by *Rasaparpam* and standard drug ascorbic acid at concentrations at 1.25µg/ml.

This indicated that % of inhibition increased with increase in concentration of both the standard and *Rasaparpam* extract. But the *Rasaparpam* extract has lower DPPHscavenging activity than that of standard. From the present study, it was concluded that the *Rasaparpam* extract has good anti-oxidant activity at higher concentrations.

It is known that oxidative stress induced cell damage not only through damage to proteins, lipids and DNA. It may also alter signaling pathways redox sensitive to changes involved in the response of apoptosis. The antioxidants are currently the subject of many studies because, in addition to some interest in the preservation of comestibles, they could be useful in the prophylaxis and treatment of diseases in which oxidative stress is implicated. Many studies realized on natural products have proven that they are especially phenolic compounds who are responsible for their antioxidant activity.

Several studies have shown the link between the traditional drug formulations rich in anti-oxidants and the incidence certain diseases such as **cancer**, diabetes, heart disease and other diseases related to aging. Phenolic compounds could prevent cancer by the action antioxidant and the modulation of several functions of proteins. Phenolic compounds can prevent carcinogenesis by affecting the molecular events in the triggering, promotion and progression stages.

Here, the reactive oxygen species (ROS) may be the triggers apoptotic process. In recent years numerous properties have been described about these compounds such as the ability to inhibit cell cycle, proliferation cellular and oxidative stress and induce detoxification enzymes, apoptosis and stimulate the immune system. It is therefore hypothesized that *Rasaparpam* of its anti-oxidant power could eliminate cancer cells.

28 Days Repeated oral toxicitystudy

Histopathology

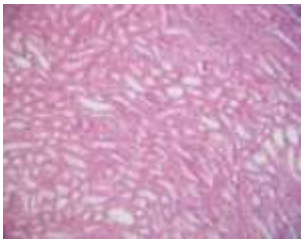
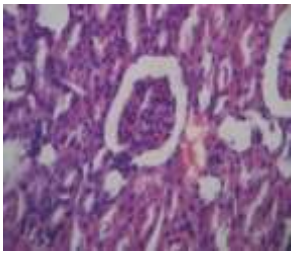
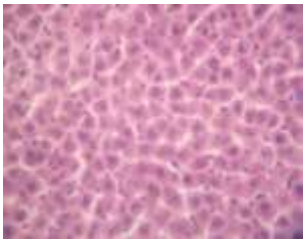
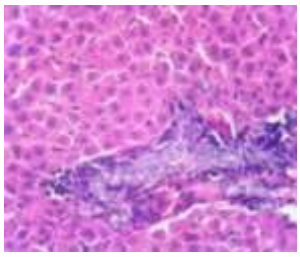
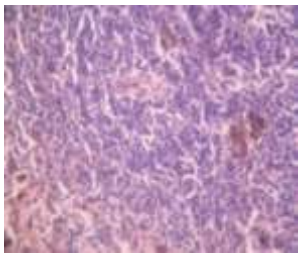
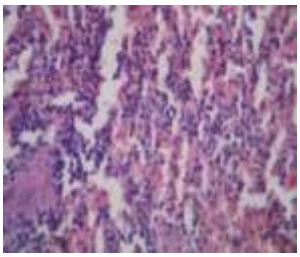
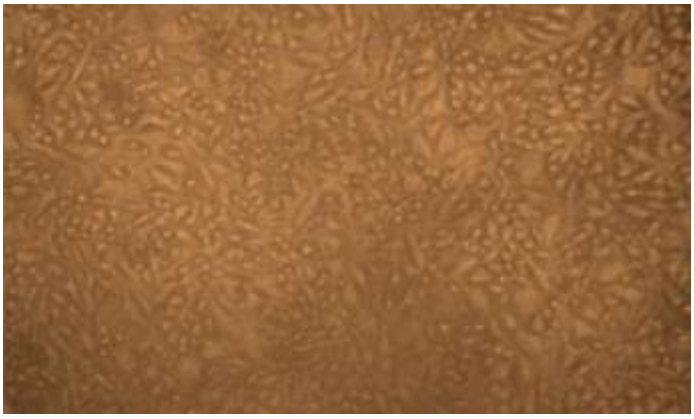
Control Group		Test Group (High Dose)
	**KIDNEY**	
	**LIVER**	
	**SPLEEN**	

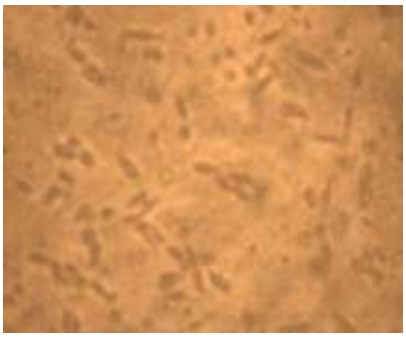
Fig.No.22. Histopathology images of Rasaparpam

Anticancer effect of *Rasa parpam* on *HeLa cell* line

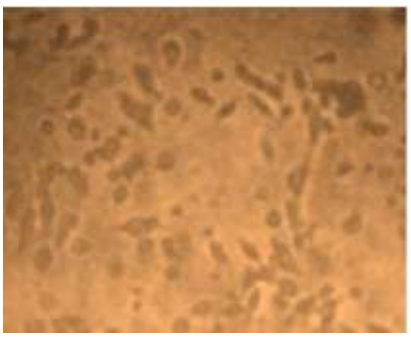
Normal HeLa cell line



Toxicity1000 µg/ml



Toxicity 125 µg/ml



Toxicity 7.8µg/ml

Fig.No.23. Anti-cancer activity of Rasaparpam.

6. CONCLUSION

Cervical cancer is the second most common malignancy found among women worldwide and some are highly resistant to radiotherapy. The other chemotherapy drugs causses intolerable side effects which are worse than the disease. This paved way for a novel anticancer drug which cures cervical cancer in a non invasive method.

- The intention of this study is to provide a solution for the above need for a non violent anticancer drug to cervical cancer. ***Rasa Parpam*** was chosen as a trial drug from the Siddha literature "***Aathma Raksha Mirtham Ennum Vaithiya Saara Sangiragam***" written by Kandhasamy Mudhaliyar which was categorized by the department of AYUSH as a classical text. Throughout the study, the safety and efficacy were tested thoroughly.
- The procedure for drug preparation and its techniques for standardization revealed GMP. The trial drug *RSP* has satisfied all parameters of testing protocol for *Parpam* which was assigned by AYUSH. It showed the accurate production and potency of *Rasaparpam.*
- Physico-chemical analysis revealed better bio-availability and richness of its mineral content. Favouring this study were the presence of inorganic matters which were found through experiments for analyzing acid and basic radicals.
- Various instrumental analysis of *Rasaparpam* such as FT-IR spectroscopy, Raman spectroscopy and scanning electron microscope demonstrated its chemical constituents, functional groups and particle size to support its indication to counter cervical cancer.
- The anti-microbial activity of trial drug was also considered for its potential
- Under OECD guidelines, the acute and 28 days repeated oral toxicity studies proved the safety of *Rasaparpam* at particular dose level. It is very useful in therapeutic dose determination.

- The pharmacological activities are justified by anticancer effect on HeLa cell lines, anti-tumour effect on HeLa and SIHA cell lines and quantitative measurement of antioxidants by DPPH assay.
- Factors like safety, efficacy, long self like, bio-availability, presence of significant elements, anions and cations and minerals favouring the activity justifies the main perspective of this study.
- Anti-cancer effect could be validated scientifically. Due to its Non-toxic anti-cancer effect, it would benefit the female community in the world.

7. SUMMARY

Trial drug ***RASA PARPAM*** was selected from the classical Tamil literature "**Aathma Raksha Mirtham Ennum Vaithiya Saara Sangiragam**" written by Kandhasamy Mudhaliyar forits anti-cancer activity.

The dissertation was started with an introduction explaining about the Siddha concept, prevalence of cervical cancer and role of the test drug in treating cancer cervix.

Review of literature in various categories was carried out. It was elaborated under Gunapadam and Modern aspect of ingredients, Siddha and modern aspect of that disease, pharmaceutical aspect and pharmacological aspect in both Siddha and modern.

All the ingredients were identified and authenticated by experts.

The compound was prepared properly by given procedure in an appropriate situation. The end product underwent standardization parameters in Siddha.

The drug was subjected to analysis such as physiochemical, biochemical and instrumental which provided the key ingredients present in the drug thus it accounts forthe efficacy of the drug.

The sample was also analysed for anti-microbial load to ensure its accuracy.

For the study protocol, required animals were approved by the IAEC under CPCSEA.

Toxicological study was made according to OECD guidelines comprising both acute and repeated oral dose 28days toxicity studies in wistar albino rats. It showed the safety of the drug which attributes its utility for long time administration.

Pharmacological studies were completed. It proved the

Anti-cancer activity through HeLa cell line , anti-tumor activity through HeLa and SiHa cell line models and anti-oxidant activity by DPPH assay of *RASA PARPAM*.

Results and discussion gives the essential validations to prove the potency of the drug.

Conclusion gives a compiled form of the study and explains the synergistic effect of all the key ingredients and activities that supports the study.

8. FUTURE SCOPE

Trial drug for the study ***RASA PARPAM*** was taken from the classical Siddha literature ***Aathma Raksha Mirtham Ennum Vaithiya Saara Sangiragam,*** Written by **Kandhasamy Mudhaliyar.**

Its validation for its Anti-cancer nature was completed at preliminary level. The result enhanced and assured its Anti-cancer property against cervical cancer.More specific experiments on animal models and also clinical trials are required to understand the exact molecular mechanisms of action. So it could be used worldwide in treatment of cervical cancer and satisfy the safe and painless anti-neo plastic treatment.

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This Certificate is awarded to Dr/Mr/Mrs.....G.:.Deepa.....

for participating as ~~Resource Person~~ / Delegate in the Eighteenth Workshop on

" RESEARCH METHODOLOGY & BIOSTATISTICS "

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16.6.2017

CERTIFICATE

Name of the student: Dr. G. Deepa, III year PG student, Gunapadam, Government Siddha Medical College, Arumbakkam, Chennai-600 106.

Name of the sample: Rasa Parpam

Name of the Experiment	Value
Loss on drying(at 105°C)	1.0 %
Total ash	99.0 %
Water soluble ash	5.40 %
Acid insoluble ash	3.65 %
pH value (10%)	6.9

(R. Shakila)
Research Officer (Chemistry) & Head,
Department of Chemistry

16/6/17.

(Dr. P. Sathiyarajeswaran)
Assistant Director (Siddha) I/c

डॉ. पी. सत्तियराजेस्वरण/Dr. P. Sathiyarajeswaran
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CERTIFICATE

CERTIFICATE

This is to certify that the project entitled, **Toxicological and Pharmacological study on RASA PARPAM & PAAVATTAI POO *(Pavetta indica - flower)* KUDINEER** in rats submitted in partial fulfilment for the degree of **M.D. (siddha)** was carried out at C.L. Baid Metha college of Pharmacy, Chennai-97, in the Department of Pharmacology during the academic year of 2016-2017. It has been approved by the **IAEC No: IAEC/XLVIII/03/CLBMCP/2016**

Dr.P.Muralidharan

IAEC Member Secretary

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